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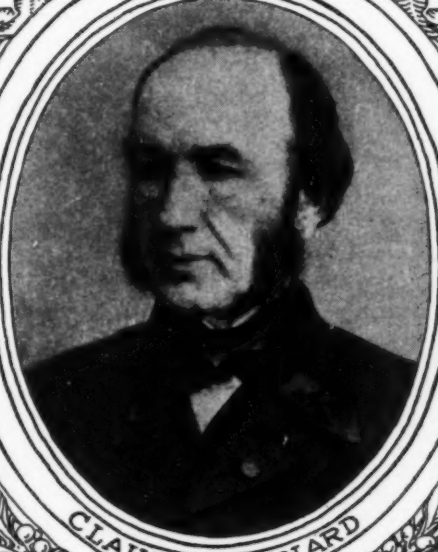

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Woodland Avenue and Thirty-sixth Street, Philadelphia, Pa.

IRON BALANCES ON FOUR NORMAL PRE-SCHOOL CHILDREN [1]

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(Received for publication September 9, 1940)

The thirty-five iron balances on normal pre-school children reported in this paper were a portion of the data collected in a study to determine the effect of a farina fortified with irradiated yeast on the mineral metabolism of the subjects. In the original plans for this study, interest centered around the effect of the vitamin-D-fortified farina on calcium and phosphorus utilization.[2] As the plans progressed, however, it was decided to take all the necessary precautions in managing the subjects and in collecting the specimens to allow for complete iron balance determinations. This was considered advisable because the period of observation was longer than any previously reported on this age group, as well as because possible interrelationships between iron and the vitamin D supplement might be observed.

EXPERIMENTAL PROCEDURE

The four children cooperating in this study were in fairly good nutritional condition and in good general health at the beginning of the study. There were three boys, O, R, and K in

[1] Published from the Michigan State College Agricultural Experiment Station as paper no. 464, new series.

The preliminary phases of this study were conducted in the Nutrition Laboratory of the Department of Home Economics of The University of Chicago; the expense of these portions of the work was defrayed by a grant from the Quaker Oats Company, Chicago.

[2] Calcium and phosphorus balances are now being prepared for publication.

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the group, and one girl, M. Subject O was 3 years old, 100 cm. tall and weighed 17 kg. R was also 3 years old, 100 cm. in height, and weighed 16 kg. Subjects K and M were 5 years old. K was 114 cm. tall and weighed 20 kg., while M was 112 cm. in height and 21 kg. in weight. At the beginning, mid-point, and end of the study, medical and physical examinations were given the children by Doctor Grissom, a member of The University of Chicago Nursery School staff. Hemoglobin percentages and red cell counts were recorded for each child.

The management of the subjects was carefully planned and supervised in every detail including an effort to prevent the intake of any form of iron from toys or other items in the environment so that intake of food iron would represent the total consumption of that mineral. No serious disciplinary problems or emotional disturbances arose to affect the progress of the study.

The customary diets of the subjects were reasonably adequate, as far as could be determined. They contained liberal quantities of milk and vegetables as well as some cod liver oil or viosterol as a supplement. During the experiment the diet was planned in a 7-day unit. An example of O's diet, with quantities of foods he ate, is given in table 1. This diet had a calculated average nutritive value of approximately 1300 calories, 45 gm. of protein, 1 gm. of calcium and of phosphorus, and 6 mg. of iron (Waller, '38). Using the average weight of O throughout the study, the calories averaged 76.3 per kilogram of body weight per day while the protein allowance was 2.63 gm. and represented about 13% of the total calories. Of the vitamins, the food furnished about 5000 I.U. of vitamin A, 75 mg. of ascorbic acid, 0.8 mg. of thiamin, and 1.5 mg. of riboflavin. Such quantities of these vitamins adequately meet the daily allowances suggested for children of this age by Stiebeling and Phipard ('39). The daily average vitamin D content of the unfortified basal diet was only about 40 I.U. In calculating this figure, minimum values for foods as given by Chaney and Ahlborn ('39) were used, because the

foods were winter foods. Each day the diet contained approximately 1 ounce (28 to 30 gm., dry weight) of either plain farina or farina fortified with a fraction of 1% of irradiated yeast. The inclusion of fortified farina resulted in amplifying the basal diet with approximately 120 I.U. of vitamin D per day. No additional vitamin or mineral supplements were given.

TABLE 1

Food list, in grams, for subject O for 7 days

EVERY DAY		DAY 1 ADDITIONS		DAY 2 ADDITIONS		DAY 3 ADDITIONS	
Milk	720	Egg	50	Bacon, cooked	10	Stew (beef)	75
Orange juice	100	Beef, ground	30	Beef, ground	60	Tomato-soup	100
Farina, cooked	140	Tomatoes, canned	60	Spinach	40	Spaghetti	25
Bread, whole wheat	20	Potato, baked	40	Peas, creamed	75	Peaches, canned	90
Crackers, graham	14	Mousse, fruit	45	Potato, baked	20	Prunes, stewed	100
Butter	24	Apple sauce	100	Pears, canned	90	Apple, E.P.	50
Sugar	10			Banana, E.P.	60		
				Apple, E.P.	50		
DAY 4 ADDITIONS		**DAY 5 ADDITIONS**		**DAY 6 ADDITIONS**		**DAY 7 ADDITIONS**	
Bacon, cooked	10	Liver	30	Bacon, cooked	10	Stew (beef)	75
Spaghetti with beef	100	Vegetable soup	150	Salmon, canned	25	Peanut butter	9
Cabbage	50	Crackers, soda	30	Potato, baked	20	Bread, whole wheat	10
Apricots	45	Beans, green	50	Beets	40	Beans, green	50
Jello	60	Potato, baked	20	Carrots	50	Peaches, canned	90
		Pears, canned	90	Pineapple, canned	60	Prunes, stewed	100
		Apple sauce	100	Apple, E.P.	80		
				Banana, E.P.	30		

The diets of the other children contained these same kinds of food with minor adjustments in quantities to meet each child's needs. All children received milk, orange juice, and farina in the quantities listed in table 1. The food intake was kept as constant as possible for each subject and every necessary precaution for procuring a uniform food supply was exercised. All food combinations were made by weighed

recipes; and all foods were prepared in enamel or aluminum utensils with plated cutlery. Distilled water was used for cooking and drinking.

After a preliminary period of 3 days which permitted the establishment of a smooth running routine, the weighed diet and the collections were begun. The experimental calendar was made up of nine 7-day periods, a total of 63 days, beginning the latter part of December, 1932, and extending into February, 1933. Three subjects, O, R and M, served for all nine periods, while subject K served for eight of the periods. One 3-year-old and one 5-year-old, O and M respectively, began the experiment on diets containing 1 ounce per day of fortified farina. After remaining on this food for 21 days (three 7-day periods) they were given the basal diet with plain farina instead of the fortified product for 21 days, then returned to the original diet for 21 days. The other two subjects, R and K, reversed the type of diet in corresponding periods, that is, they began with 21 days on plain farina, followed with 21 days on fortified farina, and finished with 21 days on plain farina again.

For chemical analysis, one-tenth of the quantity of every food eaten was made into a "composite" for each subject and at the same time, all urine and feces for corresponding periods were saved. The food aliquots, as well as the feces acidified with iron-free hydrochloric acid and mixed with some alcohol, were dried to a constant weight in porcelain or Pyrex dishes at 80°C. Precautions were taken to keep these samples covered with parchment paper caps to protect them from dust and other sources of iron contamination during the drying. When dry, they were ground in porcelain mortars to homogeneous mixtures and stored in glass-topped bottles. The urine was preserved with the aid of 0.5% of iron-free hydrochloric acid and a thin film of toluene on the surface.

Aliquots of food, feces, and urine composites were dry ashed in duplicate in an electric muffle furnace at a temperature below dull red heat. The thiocyanate method as described by Stugart ('31) with some of the modifications sug-

gested by Leverton ('38) was employed for determining the iron in these ashes. All determinations were made colorimetrically on two aliquots from duplicate ash solutions, making a total of four readings on each unknown. In this report, results on the two ash solutions are used only when they agreed within approximately 5%.

In order to determine whether or not iron metabolism was changed by substituting for 1 ounce of ordinary farina an equal quantity of fortified farina containing 120 I.U. of vitamin D per day, certain tests for significant differences in average storage of iron on the two diets were applied. These included, first, the use of Fisher's extension of "Student's" t-test as developed by Brandt ('38) for biological experiments involving reversal or switchback trials with only one pertinent source of variability. In this case, a single change in character of basal diet was imposed on the subjects. This test was supported further by the use of an analysis of variance (Baten, '38).

DISCUSSION OF RESULTS

The medical records of the children during the period of study indicated gains in weight ranging from 6 ounces for K to 2 pounds and 3 ounces for M; and in height, from about ½ inch for K to 1.7 inches for R during the 2-month period. With the exception of K's cold in period VII, when no data were collected for him, there were no illnesses or upsets which interfered with the experimental program. It will be noted that K made the poorest growth of any of the children during these 2 months.

Since this study was undertaken, the limitations of the balance technique as a means of studying iron utilization, and the unquestionable value of hematologic methods in the objective measurement of iron metabolism have been increasingly recognized. It is regretted that more extensive and accurate data on the condition of the blood of these children are not available. The results of estimations by comparison with the Tallqvist scale indicated that the hemoglobin levels of the

children were in the neighborhood of 80% throughout the study. Both of the 3-year-olds showed a slight increase in hemoglobin during the experiment and none of the children showed a decrease. The red cell counts ranged from 6.59 $\overline{M}$ to 4.88 $\overline{M}$, and decreased slightly in each child during the study. The counts were unusually high in all children at the beginning and in no case reached a figure as low as 4.65 $\overline{M}$, which is the mean value given in Guest's findings on 278 samples of blood from children 2¼ to 5½ years old (Guest, '38). On the basis of these rather limited data, it probably could be said that the children were in at least "average" condition throughout the study as far as their blood pictures were concerned.

The t-value as determined by the Brandt equations as well as the F-values obtained by analysis of variance showed that there was no significant difference in iron storage on the two types of diets. Accordingly, the results from the entire 63-day period for each child have been considered as a single series and are given in table 2.

Iron intakes. The iron intakes of the children were probably as uniform from period to period as could be expected in this type of study. Nevertheless, the percentage of variation in intake ranged from 54% for O to 28% for K. These percentages were calculated by dividing the difference between the lowest and highest intakes by the lowest intake. Expressed in milligrams per day, the average intakes ranged from 5.38 to 5.95, with an average for all children of 5.64. The level of intake ranged from 0.22 mg. for M to 0.42 mg. for O and averaged 0.31 mg. per kilogram of body weight. This would be considered a low level of iron ingestion when it is compared with the dietary allowances recommended by those who have made earlier studies of iron balances in children. Leichsenring and Flor ('32), for example, found that children retained iron in appreciable amounts on an intake which was about 0.19 mg. per kilogram, but that they retained over twice as much of the mineral on an intake of about 0.37 mg. They added the average excretions on the two levels of intake

TABLE 2

Average iron balances, in milligrams per day, from nine 7-day periods on four children

SUBJECT	DIET	PERIOD	INTAKE		EXCRETION				RETENTION	
			Total	Per kilogram	Urine	Feces	Total	Per kilogram	Total	Per kilogram
			mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
		1	7.04	0.42	0.13	4.80	4.93	0.29	2.11	0.13
	Fortified farina	2	6.23	0.37	0.17	3.81	3.98	0.24	2.25	0.13
		3	6.13	0.36	0.14	4.46	4.60	0.27	1.53	0.09
		4	4.63	0.27	0.12	4.26	4.38	0.26	0.25	0.01
O	Plain farina	5	5.23	0.31	0.24	4.11	4.35	0.26	0.88	0.05
		6	5.03	0.29	0.15	3.32	3.47	0.20	1.56	0.09
		7	5.14	0.30	0.21	3.75	3.96	0.23	1.18	0.07
	Fortified farina	8	4.57	0.27	0.19	3.59	3.78	0.22	0.79	0.05
		9	4.98	0.29	0.22	4.71	4.93	0.28	0.05	0.01
	Mean		5.44	0.32	0.17	4.09	4.26	0.25	1.18	0.07
		1	5.96	0.38	0.13	4.16	4.29	0.27	1.67	0.11
	Plain farina	2	5.23	0.33	0.24	4.16	4.40	0.28	0.83	0.05
		3	4.98	0.31	0.14	3.82	3.96	0.25	1.02	0.06
		4	5.14	0.32	0.12	3.82	3.94	0.25	1.20	0.07
R	Fortified farina	5	5.61	0.35	0.27	4.29	4.56	0.28	1.05	0.07
		6	5.96	0.37	0.12	3.64	3.76	0.23	2.20	0.14
		7	6.08	0.37	0.21	4.51	4.72	0.29	1.36	0.08
	Plain farina	8	4.47	0.27	0.22	3.43	3.65	0.22	0.82	0.05
		9	5.01	0.31	0.18	4.70	4.88	0.30	0.13	0.01
	Mean		5.38	0.33	0.18	4.06	4.24	0.26	1.14	0.07
		1	6.16	0.30	0.23	4.23	4.46	0.22	1.70	0.08
	Fortified farina	2	5.42	0.26	0.26	4.93	5.19	0.25	0.23	0.01
		3	7.22	0.35	0.19	4.13	4.32	0.21	2.90	0.14
		4	5.21	0.25	0.18	3.12	3.30	0.16	1.91	0.09
M	Plain farina	5	5.44	0.26	0.22	4.40	4.62	0.22	0.82	0.04
		6	6.47	0.30	0.36	4.13	4.49	0.21	1.98	0.09
		7	5.73	0.27	0.24	4.42	4.66	0.22	1.07	0.05
	Fortified farina	8	4.72	0.22	0.31	4.35	4.66	0.22	0.06	0.00
		9	5.80	0.27	0.23	5.00	5.23	0.24	0.57	0.03
	Mean		5.80	0.28	0.25	4.30	4.55	0.22	1.25	0.06
		1	7.06	0.35	0.15	5.24	5.39	0.27	1.67	0.08
	Plain farina	2	6.02	0.30	0.17	5.41	5.58	0.28	0.44	0.02
		3	5.58	0.28	0.14	4.09	4.23	0.21	1.35	0.07
		4	5.51	0.27	0.13	3.49	3.62	0.18	1.89	0.09
K	Fortified farina	5	6.03	0.30	0.31	5.26	5.57	0.28	0.46	0.02
		6	5.91	0.29	0.12	3.54	3.66	0.18	2.25	0.11
		8	5.92	0.30	0.15	3.77	3.92	0.20	2.00	0.10
	Plain farina	9	5.62	0.28	0.25	4.29	4.54	0.23	1.08	0.05
	Mean		5.95	0.30	0.18	4.38	4.56	0.23	1.39	0.07
O and R mean			5.41	0.33	0.18	4.07	4.25	0.26	1.16	0.07
M and K mean			5.87	0.29	0.21	4.34	4.55	0.22	1.32	0.07
All			5.64	0.31	0.20	4.20	4.40	0.24	1.24	0.07

to arrive at a "requirement" for maintenance and growth of 0.32 mg. per kilogram. Then they still further added, because of individual differences in children and because of the question of availability of iron in food, a 50% margin of safety, arriving at a standard dietary allowance of 0.48 mg. per kilogram per day. Daniels and Wright ('34) found that diets containing 0.75 mg. of iron per kilogram resulted in no higher retentions than those containing 0.59 or 0.65 mg. and concluded that approximately 0.60 mg. per kilogram would meet the needs for maintenance and growth in normal pre-school children. Both of these groups of workers have suggested

TABLE 3

Summary of iron intakes and retentions, in milligrams per kilogram of body weight, from available studies of pre-school children

AUTHORS	YEAR	NUMBER OF SUBJECTS	DAYS IN BALANCE PERIODS	INTAKE PER KILOGRAM	RETENTION PER KILOGRAM
Rose et al.	1930	1	9	0.33	—0.08
Leichsenring and Flor	1932	4	5	0.19	+0.07
		4	5	0.37	+0.18
Daniels and Wright	1934	8	5	0.63	+0.18
Ascham	1935	6	15	0.59	+0.07
This study	1940	4	63	0.31	+0.07

dietary allowances considerably above that received by the children in this study. Reference to table 3, in which data from previous studies on iron balances are briefly summarized, shows that the average level of iron ingestion in this study was about one and a half times the lowest intake reported (Leichsenring and Flor, '32) and equivalent to about half of the highest intakes (Daniels and Wright, '34).

Iron excretions. An inspection of the bottom row of figures in table 2 which represents an average of all the data collected on the four children, shows that the usual paths of excretion accounted for a loss of about 78% of the iron ingested. Urinary iron was almost negligible in quantity. For these four subjects the total output averaged only 0.20 mg. per day. Total

fecal iron averaged 4.20 mg. per day, with a fairly wide range of from 3.12 mg. to 5.41 mg. This resulted in an average total daily output of 4.40 mg. (4.20 + 0.20). From the figures on excreted iron for individual 7-day periods, however, it can be seen that there was a rather wide variation in the total quantity of iron lost from the body, the excretion of iron varying from 3.30 to 5.58 mg. per day. The percentage variation was 52% for M, 42% for O, 33% for R and 31% for K. These variations are of the same order of magnitude as those in the intake levels. When the data from all periods, however, are averaged, it is evident that the total daily output of iron varied less than 0.5 mg. among the children. Recent work on the iron metabolism of healthy adults indicates that the body has little capacity for excreting iron (Widdowson and McCance, '37). It seems that such a finding precludes the use of levels of excretion of iron as a basis for the estimation of requirement. Excretion can be considered of only secondary importance in determining needs, and fecal iron simply represents iron which, for some reason, was not absorbed.

Iron retentions. The iron retention of these children averaged 1.24 mg. per day or 0.07 mg. per kilogram. When individual 7-day balances are examined it may be observed that none of the balances were "negative," although some of the retention figures undoubtedly represent equilibrium or quantities of iron within the limits of experimental error. If a balance of 0.02 mg. per kilogram or less is considered as practical equilibrium, this state of very low or negligible retention existed during one or two 7-day periods for each of the children during the study. On the other hand, rather high retentions of 0.10 mg. or more per kilogram per day were found in an equal number of periods for all children. An average of only 22% of the total intake was stored by these growing children. Further, the retention of iron varied considerably from period to period. Of course, in some individual periods the level of retention was directly proportional to the level of intake, that is, a low storage on a slightly lowered intake or a high storage on a slightly increased intake; but

this was only true in approximately 40% of the balances. Correlation of retention with intake was more evident in subjects O and M than in R and K.

Recognizing that lack of uniformity in techniques employed in balance studies makes comparisons of any two of them difficult, it may be of interest, nevertheless, to examine the storage of iron on some levels of intake which have been reported in other studies, as summarized in table 3. If, first, storages on intakes very similar to the intakes of the children in this study are compared, it will be observed such storages vary from —0.08 mg. in the one child studied by Rose et al., to 0.18 mg. per kilogram in the four children studied by Leichsenring and Flor, the average retention of 0.07 mg. in this study representing about a mid-point between these two extremes. If next, the intakes which permitted retentions identical with the average retention in this study are compared, it is seen that these intakes varied from 0.19 mg. to 0.59 mg., the intake of this study averaging 0.31 mg. In other words, there is no consistent level of storage on a given intake. Probably previous nutritional status with regard to iron is a much more important factor in determining the absorption and utilization of iron from food than any observation which can be made during the course of a controlled balance study. It is evident from the summary of the findings of other investigators, as well as from data in this study, that growing children usually are absorbing some iron from their food for maintenance and growth.

The length of this study makes the average retention levels of somewhat more interest, perhaps, than those observed during shorter periods. Nevertheless, the interpretation of the utilization of iron in terms of the adequacy of the intake is difficult to make and possibly is not entirely justified without a more complete history of the hematopoietic processes which took place during the period of observation. One or two points, however, may be mentioned. The average level of storage of iron from the adequate and varied combination of foods in the diet was surprisingly uniform in all the

children. Within these averages, however, there were wide variations in retention, ranging from a state of practical equilibrium to levels which would be considered high. If an unbiased interpretation is made on the data of this study, an unprecedented conclusion might be reached. For instance, since the subjects seemed to be in good physical condition at the beginning and throughout the study, with blood pictures which were reasonably acceptable, and since the diet was judged adequate in other essential nutrients such as protein, calcium, phosphorus, and certain of the vitamins, it might be concluded that the children were using as much dietary iron as they needed during the course of the investigation. Certainly the wide variations in period to period retentions support such a statement. In other words, variations in retention of iron during the 2-month period reflect variations in metabolic demand, with the retention of considerable amounts of iron at times, and with almost negligible storage at other times. On the other hand, a more conservative viewpoint would still direct toward the advisability of providing liberal quantities of iron in food for children. The surprisingly uniform average storage of iron by all the children, and certain indications from the limited hematologic records, may mean that the children made as good utilization of iron as the particular diet permitted. Further, these data do not preclude the possibility that the subjects would have been in better nutritional condition with regard to iron had they had a different diet and/or a higher level of iron intake. It will probably appear to some readers, therefore, that the suggestion of the adequacy of the rather low iron intake fed the children in this study is of doubtful propriety, especially when the many hazards to which iron is subject in the course of digestion and assimilation are taken into consideration. Perhaps these findings are best used as a basis for the statement that great anxiety over the need for providing impractically high levels of iron in children's diets is unwarranted. In the future, studies of iron metabolism, particularly if directed toward the establishment of dietary allowances, should include data on

utilization of iron within the body, since accumulating evidence indicates both the efficient use of absorbed iron and the low capacity for excretion of iron. At the moment, measures of blood changes correlated with balance studies hold the greatest promise as criteria for iron utilization.

SUMMARY

Thirty-five iron balances on four normal pre-school children, 3 and 5 years old, are reported in this study. For 63 days, which were divided into nine 7-day experimental periods, the children received daily a mixed diet furnishing an average iron content of 5.64 mg., or 0.31 mg. per kilogram of body weight.

On this iron intake varying amounts of the mineral were stored, ranging from a state of practical equilibrium to 2.90 mg. of iron per day. The average iron retention by all children throughout the study was 1.24 mg., or 0.07 mg. per kilogram of body weight per day.

The average level of retention represented about 22% of the intake of iron.

The replacement of 1 ounce of plain farina in the basal diet with an equivalent quantity of farina fortified with irradiated yeast containing 120 I.U. of vitamin D had no significant effect on iron retentions.

Grateful acknowledgment for their willing assistance is made to all those who helped with this study, especially to Mrs. R. Heninger and Mrs. J. Vorres and their children; to Frederick Fletcher, Marguerite Scott and Dr. Louise McCluskey who were laboratory assistants; and to Dr. Marie Dye, Dean of Home Economics, Michigan State College and Dr. Lydia J. Roberts, Chairman, Home Economics, The University of Chicago, for kind direction and advice.

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THE EFFECT OF DRY HEAT UPON THE ANTICATARACTOGENIC QUALITY OF CERTAIN PROTEINS[1]

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The incidence and rate of cataract development in rats fed galactose are increased by an inadequate amount of protein and inhibited by a high protein ration. These findings were reported by Mitchell and Cook ('37, '38 a, b) and partially confirmed by Yudkin and Geer ('40). The comparative effects of different types or sources of protein in such rations were reported by Mitchell and Cook ('38 a). Lactalbumin, beef muscle, cod fish, beef fibrin, soy bean and wheat gluten, fed on an equivalent nitrogen basis, gave approximately the same degree of protection or slightly less and egg albumin somewhat more protection than casein. The differences were not great, and certainly the anticataractogenic quality did not parallel the growth value of these proteins.

Mitchell, Cook and Henderson ('40) reported work with several nitrogenous factors other than protein. Since an enzymic hydrolysate of casein gave almost the same degree of protection as the nitrogen equivalent in commercial casein, it was evident that the intact protein molecule was not an essential factor. When cystine and methionine were reinvestigated with more accurate feeding technique than in earlier work, they

[1] Contribution no. 375 of the Massachusetts Agricultural Experiment Station, from the Department of Home Economics Research.

[2] Most of the data reported in this paper were presented by Mary D. Henderson in partial fulfillment of the requirements for the degree of Master of Science, Massachusetts State College.

showed little if any inhibitory effect. Urea and choline, representative of simpler nitrogenous compounds, gave negative results.

Another approach to the problem was the investigation of the effect of proteins modified by physical or chemical means so as to alter their nutritive values. Chick et al. ('35) reported that casein suffered a marked loss in biologic value due to prolonged exposure to dry heat. Morgan et al. ('30, '31) had previously shown that less drastic heating lessened the growth value of both cereal proteins and casein. Thus the question arises: does the type of treatment which affects the growth value of protein also affect its cataract-inhibiting quality?

The data presented in this paper show the effect of prolonged exposure to dry heat upon the growth value and anticataractogenic quality of four proteins—casein, egg albumin, wheat gluten and beef fibrin—when incorporated into rations containing galactose.

EXPERIMENTAL PROCEDURE

The four proteins used were chosen as representative of distinct types and were easily available from commercial sources. A drastic heat treatment which might be expected to alter the growth value of the protein was obviously necessary in order to answer the question raised, and yet it was essential not to sacrifice edibility if the experiment were to be successful. In the present series of tests the raw dried commercial egg albumin was fed both as purchased and in the coagulated form previously used in this laboratory. The latter was prepared by dissolving the raw product in a large volume of warm water and coagulating with live steam. The filtered coagulum was spread on trays, dried at low temperature in circulating air and ground to a coarse powder. The proteins were heated as follows: Weighed amounts were spread in 1-inch layers in glass trays and heated for 96 hours in an electric oven at 125°C. Each batch was thoroughly stirred at 24-hour intervals. Each lot of protein was sufficient for the entire experiment and was divided into two portions—one unheated and

the other heated. Nitrogen determinations on each form served as the basis for calculating the protein content as shown in table 1.

The formulas for the experimental rations were calculated to provide 15% of protein. Cornstarch was added in different amounts according to the amount of the crude protein used. The skeleton formula for the galactose ration had the following percentage composition: galactose, 25; protein 15 (actual amount of crude protein calculated from figures in table 1); hydrogenated cottonseed oil,[3] 9; cod liver oil, 2; salt mixture no. 351,[4] 2.5; brewers' yeast, 5, and cornstarch to make up to 100%.

TABLE 1

PROTEIN	COMMERCIAL OR UNHEATED FORM			HEATED 96 HOURS AT 125°C.		
	Nitrogen	Conversion factor	Protein	Nitrogen	Conversion factor	Protein
Casein	14.25	× 6.38	= 90.9	14.92	× 6.38	= 95.17
Egg albumin, raw	13.25	× 6.38	= 84.5			
Egg albumin, coagulated	14.04	× 6.38	= 89.6	14.75	× 6.38	= 94.0
Wheat gluten	14.29	× 5.7	= 81.46	15.05	× 5.7	= 85.95
Beef fibrin	14.55	× 6.25	= 90.95	15.15	× 6.25	= 94.71

A second series of rations was made like the first in all respects except that the galactose was omitted and all the carbohydrate was furnished by cornstarch. The rats fed the galactose-free diets served as controls with respect to the effect of heat upon the growth value of the proteins when cataract formation was not a factor.

The experiment was planned to make the following comparisons possible: (1) the effect of dry heat treatment upon the growth value of proteins fed in a ration containing starch as the source of carbohydrate (table 2); and (2) the effect of dry heat treatment upon the growth and anticataractogenic values of proteins fed in a ration containing 25% of galactose comparing, first, the plain vs. heated protein (table 3, part 1)

[3] Crisco.

[4] Hubbell, R., L. B. Mendel and A. J. Wakeman, 1937, J. Nutrition, vol. 14, p. 273.

and, second, three unheated proteins and three heated proteins (table 3, part 2).

The "paired" feeding method, used throughout this experiment, was necessary not only to insure a comparable intake of protein, but also an equal intake of the cataract-producing agent, galactose. In previous work it has been noted that a lower food consumption may greatly delay the onset of cataract because of the reduced intake of galactose, and not because of any protective nature of the ration.

The variation in litter susceptibility to cataract was also a factor in planning experiments. Litter mates of the same sex, as far as possible, were grouped for paired feeding, and at least three litters of rats were used in making each comparison. Rats from a susceptible strain were used throughout, and were started on the experimental rations at 25 or 26 days of age.

A weekly ophthalmoscopic examination of the eyes was made. A drop of a 0.2% solution of atropine sulphate, administered about 15 minutes beforehand, served to dilate the pupils completely. The slight changes thus recorded served as a warning of more advanced changes or, in some cases, became the only criterion for judging the extent of the damage to the lens when mature cataract failed to develop. The first appearance of a definite posterior opacity visible to the naked eye was recorded as "mature posterior" and the number of days on the experimental ration until this type of opacity appeared has been used as the time figure for measuring the rate of cataract development. Failure to show visible opacities at the end of the experimental period of 8 or 10 weeks was recorded as "immature," even though advanced changes visible with the ophthalmoscope had been present for many weeks in most cases. This procedure is explained in detail because different laboratories are using various standards for the development of cataract, and this fact may lead to some confusion unless resulted are carefully interpreted.

Blood sugar determinations, using the Benedict micro method, were made on most of the rats during the period of cataract development and upon control animals during the

same period. From one to three observations were made on each rat and averaged as representative of that animal. A high blood galactose superimposed on a relatively normal amount of glucose was regularly found in rats on galactose rations. It seemed desirable to determine whether there might be any significant alteration in the total blood sugar level with the various forms of protein, especially in those cases where there appeared to be a difference in the protective action against damage to the lens.

TABLE 2

The effect of dry heat upon the growth value of proteins when fed in a starch ration

PAIRED FEEDING OF RATIONS CONTAINING 15% OF PROTEIN AS		NUMBER OF RATS	AVERAGE GROWTH IN 3 WEEKS	AVERAGE FOOD INTAKE IN 3 WEEKS	AVERAGE BLOOD SUGAR
			gm.	*gm.*	*mg./100 cc.*
Casein	%				
plain	16.5	3	42	181	111
heated	15.8	3	24	181	115
Egg albumin					
raw, dried	17.7	2	35	157	128
coagulated	16.7	2	30	157	124
heated	16.0	2	34	157	129
Wheat gluten					
raw	16.4	3	22	147	126
heated	16.0	3	6	147	133
Beef fibrin					
raw	16.5	4	74	201	124
heated	15.8	4	63	201	128

RESULTS AND DISCUSSION

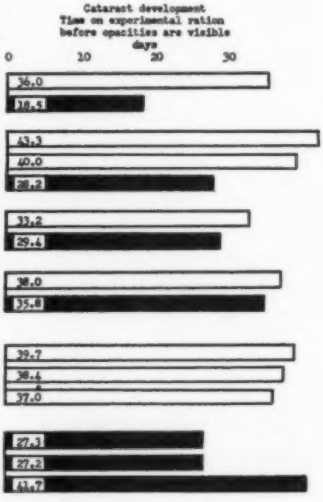
The data presented here confirm those from other laboratories concerning the loss of growth value of certain proteins when subjected to long exposure to dry heat. This loss of biologic value was evident to about the same degree with the starch rations (table 2) as with the galactose rations (table 3). The degree of heat injury to the proteins, calculated from the retardation of growth observed, was in the decreasing order, gluten, casein, fibrin, egg albumin, with the last showing little if any deterioration.

The question as to whether the order of loss in protective action against cataract would correlate with the loss in growth value is quite definitely answered in the negative. A careful study of table 3, which summarizes all results on the galactose rations, reveals ample evidence that the loss of protective action does not correlate with either the loss of growth value or with food consumption.

TABLE 3

The effect of dry heat upon the growth and anticataractogenic value of proteins when fed in a galactose ration

Paired feeding of rations containing 25% galactose plus 15% of protein as		Number of rats	Growth 3 weeks grams	Food eaten 3 weeks grams	Blood sugar mg. per 100cc.	Incidence of mature cataract percent	Cataract development Time on experimental ration before opacities are visible (days)
Casein	percent						
plain	16.5	6	46	188	225	92	36.0
heated	15.8	10	29	187	286	100	18.5
Egg albumin							
raw dried	17.7	9	46	206	240	78	43.3
coagulated	16.7	9	48	208	256	83	40.0
coag. heated	16.0	10	40	212	272	95	28.2
Wheat gluten							
raw	16.4	9	22	151	240	83	33.2
heated	16.0	10	9	151	244	95	29.4
Beef fibrin							
raw dried	16.5	12	46	201	-	83	38.0
heated	15.8	12	52	201	-	87	35.8
Untreated proteins compared:							
Casein	16.5	6	49	209	255	75	39.7
Egg albumin (coagulated)	16.7	6	52	209	242	83	38.4
Wheat gluten	16.4	6	45	209	232	75	37.0
Heated proteins compared:							
Casein	15.8	6	18	179	274	83	27.3
Egg albumin	16.0	6	22	179	273	92	27.2
Wheat gluten	16.0	6	16	179	224	67	43.7



In the first four groups in table 3 the results with heated and untreated proteins are compared, the solid bar representing the rate of cataract development on the heated protein in each case. The wheat gluten which lost the most in growth value upon heating, lost almost none of its protective action. On the other hand, egg albumin, which lost the least in growth value suffered a definite loss in protective action. Heated casein lost in both values while heated fibrin lost almost nothing in protective value and very little in growth value.

The last two groups in table 3 make possible a comparison among three of the untreated proteins and among the same three when heated. The data in the last group tend to confirm the previous conclusion that wheat gluten loses less in anti-cataractogenic value when heated than do casein and egg albumin. The lower food consumption of the heated protein rations as compared with that of the untreated protein rations adds significance to the already obvious differences. In spite of the reduced intake of galactose, two of the heated proteins allowed an increase in the rate of cataract development.

The blood sugar values of all rats on galactose rations (table 3) are pathologic, as is to be expected, and contrast sharply with those of rats on starch rations (table 2). The relatively higher values in both groups of rats fed heated casein or heated egg albumin, as compared with litter mates fed the respective untreated proteins, are suggestive of an explanation for the more rapid lenticular injuries in the former. However, the variability of the blood sugar values for several rats on the same ration and for the same rat at different times raises some doubt as to the significance of the average figures presented. Studied statistically, the differences between the plain and heated casein groups are significant, but between the raw and heated egg albumen they are questionable. Every effort was made to keep the conditions constant as to the amount of food consumed and the interval of time before the blood was drawn, but at best the sample does not always represent the maximum rise in blood sugar during absorption.

The influence of these different protein preparations upon the metabolism of galactose as measured by the level of the sugar in the blood is worthy of further consideration. In our earlier work with different quantities of casein in the ration, differences of the same order were noted. When the protein was increased from 15 to 45% the blood sugar average dropped from 276 to 213 mg. per 100 cc. of blood. At the same time the low protein (5%) group had almost exactly the same blood sugar as the medium protein (15%) group, and yet the former

developed cataract much more rapidly. Thus while the differences in blood sugar which do exist are in the right direction, yet they scarcely seem sufficient to explain the retardation or hastening of the pathologic changes in the lenses. Perhaps the lens of the young rat is particularly susceptible to damage and sensitive to slight changes in the blood sugar level. It may be the extent of fluctuation in the level of blood sugar which is injurious rather than the absolute level reached after a given amount of a ration containing galactose has been consumed.

In previous publications from this laboratory it has been suggested that the galactose in the blood stream may cause direct damage to the lens capsule, altering its permeability and thus allowing a shift in the ionic equilibrium which maintains the proteins in the lens in perfect transparent solution. The observed protective action of protein must be exerted either by lowering the level of the injurious factor in the blood stream or by increasing the resistance of the capsular membrane to injury. The failure to observe significant differences in the blood sugar level between different ration groups which did show a striking difference in the degree of lenticular injury has led us to favor the hypothesis that the protective action of the protein, in some way, concerns the lens or its capsular membrane. The degree of galactemia is undoubtedly a contributing factor. Thus, if some nitrogenous factor does influence galactose metabolism, by increasing the rate of glycogenesis in the liver, for instance, this point is worthy of further study. If the protein factor is protective in the presence of the injurious agent, galactose, it is possible that the same factor might exert a similar protection against other injurious agents in the blood stream. The nature of the protective factor in protein and its mode of action in the presence of a galactemia are still under investigation.

SUMMARY

1. Since protein appears to exert a protective action against galactose cataract in rats, the question has been raised as to whether the type of treatment which is known to decrease

the growth value of a protein will also affect its cataract-inhibiting quality.

2. Four proteins, casein, egg albumin, wheat gluten and beef fibrin, were fed as purchased and after heating for 96 hours at 125°C. Each protein was incorporated into a 25% galactose ration at a level to provide 15% of protein.

3. The growth value of these proteins was damaged by heat in the decreasing order: gluten, casein, fibrin, egg albumin.

4. The anticataractogenic property of these proteins was diminished by heating in the decreasing order: casein, egg albumin, gluten, fibrin.

5. Since the losses in the two properties of protein due to exposure to heat do not run parallel, it follows that the amino acid or group of amino acids which exert the protective action are not the same as those essential for growth.

6. In some instances the blood sugar values correlated with the more rapid cataract development but there is considerable doubt as to whether the degree of galactemia alone can account for the differences in lenticular injury.

7. It is possible that the protein factor may be protective by some local action in the eye rather than by influencing galactose metabolism.

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THE EFFECT OF THE HYDROLYTIC PRODUCTS OF CASEIN AND DEAMINIZED CASEIN ON THE CATARACTOGENIC ACTION OF GALACTOSE [1]

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Earlier investigations of the influence of the type and amount of protein and other nitrogenous factors on the cataract-producing action of galactose have been reviewed in a previous paper (Henderson and Mitchell, '41). Pursuant to the theory that a type of treatment which alters the growth value of a protein may or may not affect its anticataractogenic quality, casein deaminized by treatment with nitrous acid has been the subject of investigation. The inadequacy of deaminized casein as a source of protein, shown by Hogan and Ritchie ('34), was manifested by the anemic condition of their rats as well as by retarded growth and early death. These results seemed to indicate that during deaminization either an essential nitrogenous factor is removed, or a toxic substance is produced.

In preliminary experiments in this laboratory the fact was verified that rats could not survive when deaminized casein was the sole source of protein in the ration; when no more than two-thirds of the protein was supplied by deaminized casein

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[2] Most of the data reported in this paper were presented by Mr. Edwin L. Moore in partial fulfillment of the requirements for the degree of Master of Science, Massachusetts State College.

and the other third by commercial casein, growth was subnormal but most of the rats survived. When this combination of proteins was incorporated into a ration which contained 25% of galactose it was evident that the nitrous acid treatment had rendered the casein more protective against cataract although reducing the growth value. This finding was quite contrary to what one would expect considering the supposed loss of nutritive elements. The unpalatability of rations containing the deaminized casein made it necessary to use paired feeding in comparing the effect of the plain and treated casein. Although this procedure, of necessity, reduced the consumption of galactose and the consequent rate of cataract development in both groups, the protective action of the deaminized casein was evident, especially in the more susceptible litters.

These findings seemed to warrant some further investigation of deaminized casein and its hydrolytic products, in the hope of finding a fraction which might contain the protective factor or factors. It seemed possible that perhaps some new compound is formed in the process of deaminization, which is responsible for the increased protection observed.

The plan of the present investigation was to hydrolyze, by both acid and enzymic hydrolysis, large batches of commercial casein and deaminized casein and then fractionate the hydrolysates into the major amino acid groups. It was necessary to work with quantities sufficient to permit the feeding of the fractions to experimental animals. The preparation of the deaminized casein, hydrolysates and fractions was carried out as quantitatively as possible, with careful records of all yields and analyses of the various products for nitrogen. The formulas for the galactose rations containing the various preparations were calculated according to the yield and nitrogen content as will be explained later.

CHEMICAL PREPARATIONS FROM CASEIN

1. Deaminized casein. The procedure as outlined by Dunn and Lewis ('21) was followed. Yields averaged about 90% and were of the order obtained by the original investigators.

Analyses of the original casein and the deaminized product showed 14.04 and 14.21% nitrogen, 2.58 and 1.69% ash, and 7.46 and 4.21% moisture respectively.

2. Acid hydrolysate and fractions. In the preparation of the acid hydrolysate and its fractions the method of Dakin ('18) was employed and batches of 500 gm. each of casein and deaminized casein were used. Table 1 gives the hydrolytic

TABLE 1

Summary of casein and deaminized casein hydrolysis. Figures represent the per cent of the original nitrogen recovered. Letters are abbreviations used for subsequent reference to products prepared and used in feeding tests

INITIAL PRODUCT	TOTAL HYDROLYSATE	FRACTIONATED HYDROLYSATES					
		Humin	Barium sulfate precipitate	Insoluble solids	Monoamino acids	Diamino and dicarboxylic acids	Proline and peptides
Acid hydrolysis							
Casein C	75.45 CAH	0.19	9.83		24.86 CAHMA	25.58 CAHDD	15.50 CAHPP
Deaminized casein DC	83.82 DCAH	0.58	9.39		18.70 DCAHMA	39.04 DCAHDD	14.95 DCAHPP
Enzymic hydrolysis							
Casein C	93.93[1] CEH			4.93	21.81 CEHMA	48.73 CEHDD	—[2] CEHPP
Deaminized casein DC	90.56[1] DCEH			4.67	10.35 DCEHMA	57.12 DCEHDD	—[2] DCEHPP

[1] Nitrogen of the trypsin is not included in the figure for the total hydrolysate but could not be excluded from the figures for the fractions.

[2] A portion of this fraction was lost and therefore the per cent recovered could not be calculated.

products prepared, the abbreviations used and the percentage of the total nitrogen recovered in each fraction. The hydrolysates and fractions, other than the monoamino acid fractions, were dried on cornstarch because of their sticky consistency.

3. Enzymic hydrolysate and fractions. It is well known that acid hydrolysis is, in general, more destructive than enzymic hydrolysis. The latter process more nearly simulates the breakdown of protein in the animal body. For these reasons,

casein and deaminized casein were subjected to tryptic hydrolysis under controlled conditions as follows: 350 gm. of casein (or deaminized casein) were suspended in 3.5 liters of distilled water. To this suspension were added 28 gm. of anhydrous sodium carbonate (to produce a pH of about 8.1), 17.5 gm. of trypsin [3] and a few crystals of thymol as preservative. A thin layer of toluene was added and the flasks stoppered with cotton and incubated at about 37°C. for 3 days. On the third day another 17.5 gm. of trypsin were added to each flask and the pH again adjusted to 8.1. This adjustment was made every 3 days throughout the digestion period of 3 weeks. Finally the digestion mixture was boiled to drive off the toluene and to stop any further action of the enzyme. The mixture was then concentrated to a thick syrup in a distilling flask under reduced pressure and deposited on cornstarch and dried.

Using another 350 gm. each of casein and deaminized casein, the same procedure was followed to the point where the hydrolysate was boiled to free it from toluene. At this point the digest was filtered and the residue thoroughly washed to free it from soluble material. This residue was dried on cornstarch. The filtrate and washings were concentrated on the steam bath and were subjected to fractionation by Dakin's method. The fractions were isolated and concentrated by the same procedure as in the acid hydrolysis.

All fractions which were deposited on starch were dried in an oven at approximately 40°C. with continuous circulation of air. The resulting material was ground to a powder in a ball mill. Representative samples of each dry powder were analyzed for nitrogen in order to calculate the total nitrogen recovered in each fraction and the proportion of each fraction to use in the feeding trials. Blanks were subtracted for the cornstarch used in all preparations and for the trypsin used in the total enzymic hydrolysate. It was impossible to make correction for the trypsin in the fractions, because the percentage of trypsin remaining in each fraction could not be determined.

[3] The trypsin was obtained from Fairchild Bros. and Foster, New York, N. Y.

Comments on relative yields. A larger percentage yield of monoamino acids was obtained from casein than from deaminized casein by both methods of hydrolysis. This result was unexpected because the lysine, after deaminization of the casein, should shift from the diamino to the monoamino acid fraction if the theories as to the alteration in the molecule are correct. This finding points toward an incomplete hydrolysis of the deaminized product to the free amino acids. The relatively larger yield of diamino and dicarboxylic acids from the deaminized casein supports this theory because any soluble material not extracted in the preceding groups would appear in this fraction. The loss of nitrogen in the barium sulfate precipitate of the acid hydrolysis does not occur in the enzymic hydrolysate and thus more nearly the full value of the protein should be recovered from the latter.

EXPERIMENTAL FEEDING OF CASEIN PREPARATIONS

The ration containing 25% of galactose as the cataractogenic agent was modified with respect to the protein and cornstarch only and had the following percentage composition: casein (commercial), 5.5; galactose, 25; hydrogenated cottonseed oil,[4] 9; cod liver oil, 2; salt mixture,[5] 2.5; brewers' yeast, 5; casein, deaminized, hydrolysates or fractions, 10 (calculated according to nitrogen content or equivalent fraction); cornstarch—to make total up to 100%.

The amount of the total hydrolysate incorporated in the ration was calculated on the basis of the nitrogen equivalent of the 10% of protein fed as casein. The amount of any fraction fed, replacing the 10% of protein, was proportional to the percentage yield of that fraction from the total casein or deaminized casein hydrolysates in terms of the nitrogen content. This procedure was adopted in order that any fraction might afford as much protection but no more than its 10% protein control, because the amount of each fraction used should theo-

[4] Crisco.

[5] Hubbell, R. B., L. B. Mendel and A. J. Wakeman 1937 J. Nutrition, vol. 14, p. 273.

retically be the same in the two rations. A fraction which carried no protective action would add nothing to the degree of protection afforded by the 5.5% of casein already present in the basal ration. So far as possible the corresponding fractions prepared from the acid and enzymic hydrolysis differed only with respect to the nitrogen content of the enzyme used.

All rats used in this experiment were from one strain known to be susceptible to galactose cataract. Paired litter mates were used throughout and were started on experimental rations at 25 or 26 days of age. The number of days on experimental rations until visible opacities appeared were recorded as the time for the development of cataract. Failure to show visible opacities at the end of the experimental period of 8 weeks was recorded as "immature" with the degree of early lenticular damage estimated by ophthalmoscopic examination. A limited number of rats fed under rigidly controlled conditions was necessitated by the nature of the experiment and the small amounts of the fractions available.

The equalization of food intake between pairs or triplets insured a comparable intake of the casein preparation being tested and also an equal intake of the cataractogenic agent, galactose. Thus, the only valid basis for interpreting the data reported in table 2 is to compare the incidence and rate of cataract development in litter mate controls eating the same amount of food. The poor growth of all rats on deaminized casein products was due to a distaste for these products. Deaths due to inanition in the DCAH and DCAHDD groups made complete observations impossible. In general it was noted that the enzymic hydrolysate and its fractions were less objectionable to the rats, judged by the growth and food intake figures. Thus the results with enzymic hydrolytic products were more satisfactory than those with the acid products.

The results of the feeding tests with all of the casein preparations are summarized in table 2. Growth and food intake figures are significant to the extent that the low intakes retarded both growth and cataract development. In studying

TABLE 2

Data on rats fed casein and deaminized casein and their hydrolytic products incorporated in a galactose ration. Paired feeding with controls on casein (C) and deaminized casein (DC)

CASEIN OR ITS DERIVATIVES IN THE RATION [1]	NUMBER OF RATS	AVERAGE GROWTH IN 3 WEEKS	AVERAGE FOOD EATEN IN 3 WEEKS	MATURE CATARACT		BLOOD SUGAR
				Incidence	On exp. ration	
		gm.	*gm.*	*%*	*days*	*mg./100 cc.*
DC	16	15	106	*47*	*59.6*	225
C	10	17	106	*60*	*46.5*	233
CAH	4	—3	87	100	36.3	—
C	2	18	89	50	47.0	—
CAHMA	3	42	191	100	23.6	220
C	3	60	194	100	26.5	245
CAHDD	3	6	135	100	28.5	—
C	2	17	135	67	48.3	—
CAHPP	3	8	138	100	29.3	246
C	2	32	139	0	56.0	283
DCAH	4	All died in 9–14 days				
DC	4	All died or were moribund due to starvation				
DCAHMA	4	9	111	75	33.8	237
DC	4	4	111	50	50.0	238
DCAHDD	4	—12	89	Died due to starvation		
DC	4	0	91	Died due to starvation		
DCAHPP	4	0	97	75	36.2	253
DC	4	5	97	25	52.6	247
CEH	4	60	209	75	39.5	217
C	4	65	209	25	49.6	213
CEHMA	4	46	216	100	17.8	262
C	2	63	216	100	28.0	243
CEHDD	3	30	161	100	21.7	248
C	2	37	161	66	46.7	229
CEHPP	2	12	140	100	15.5	227
C	2	31	143	75	35.5	271
DCEH	6	10	105	*25*	*54.3*	234
DC	5	9	104	*25*	*49.8*	232
DCEHMA	5	12	113	60	35.7	227
DC	4	18	113	20	54.6	223
DCEHDD	4	5	109	*25*	*50.8*	241
DC	3	6	109	*63*	*54.5*	228
DCEHPP	2	10	122	100	19.5	239
DC	2	13	122	100	36.0	271

[1] The explanation of the letter abbreviations for casein derivatives in the rations is given in table 1.

the figures on incidence and time of cataract development it will be noted that the incidence is higher and the rate more rapid than in the controls for all preparations tested except in the groups italicized. The greater protective action of deaminized casein as compared with regular casein is evident from the first set of figures. Of the hydrolytic products only the DCEH and the DCEHDD showed a protective action equivalent to or greater than the controls. This might indicate that the protective factor present in the DCEH was concentrated in the DCEHDD fraction. There was complete lack of protective action shown by most of the other derivatives or fractions. The few instances in table 2 where the incidence of mature cataract in control groups was very low or zero were due to reduced food intake or to more resistant litters. The experimental group in each of these comparisons (CAHPP and CEH) showed a high incidence and rapid development of cataract, thus supporting the conclusion that the derivative in question was not as protective as the control. The nature and mode of action of the protective factor in deaminized casein are being further investigated.

Blood sugar determinations are significant in a study of this kind because it is desirable to know whether the protective action is exerted in the presence of a high blood sugar, or whether it works by lowering the level of sugar in the blood. These determinations were made two or three times on each rat except where a moribund condition or emaciation prevented. In general the blood sugars remained high, i.e., well above 200 mg. per 100 cc. of blood, and did not vary consistently with the rate of cataract development in comparable groups. From these limited observations it would appear that the protective agent acts in the presence of the high blood sugar rather than by influencing the metabolism of the sugar directly. This local action upon the lens or lens capsule has not been explained. Factors bearing on this point have been discussed elsewhere (Henderson and Mitchell, '41).

SUMMARY

1. Preliminary work showed that deaminized casein was more protective against galactose cataract than the commercial casein from which it was prepared.

2. Hydrolytic products of casein and deaminized casein were prepared by both acid and enzymic hydrolysis and fractionated according to Dakin's method.

3. The enzymic hydrolysate of deaminized casein was somewhat more protective than the deaminized casein from which it was prepared, when fed as two-thirds of the nitrogen of the ration.

4. Of the fractions, the diamino dicarboxylic acids afforded as much protection as the whole hydrolysate, while the monoamino and proline and peptide fractions showed no protection whatever.

5. Blood sugar determinations indicated that the protective action was exerted in the presence of the high blood sugar and not by a lowering of the blood sugar level.

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CAROTENE AND VITAMIN A IN CATTLE BLOOD PLASMA WITH OBSERVATIONS ON REPRODUCTIVE PERFORMANCE AT RESTRICTED LEVELS OF CAROTENE INTAKE[1]

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ONE FIGURE

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According to Palmer ('22), Krukenberg (1885) deserves the credit for the first extensive study of the chromolipid pigments in the blood serum of cattle. The discovery that carotene was the precursor of vitamin A in the animal body has led to renewed interest in this field. Carotene isolated from cattle serum by Euler and Virgin ('32) was shown to have vitamin A activity and these authors cite a case where, after winter feeding of an ox, the amount in the serum was greatly reduced. Fine ('33) also fed ox serum to young rats on an otherwise vitamin A deficient diet and concluded that serum contained vitamin A. Chevallier and Choron ('35) made extracts from the blood of rats, guinea pigs, rabbits, swine, sheep, horses, cattle, and humans, and on spectrographic analyses obtained absorption bands characteristic of vitamin A. Numerous investigators in recent years have reported studies on the vitamin A and carotene contents of human plasma both in health and disease. The work of Clausen and McCoord ('38) in which they have used a modification of the Carr-Price antimony trichloride method for the determination of vitamin A is especially notable. European medical investigators have published

[1] Presented at the meetings of the American Chemical Society, Cincinnati, Ohio, April 8–12, 1940.

considerable material in this field. For example, the work of Wolff ('36) and Menken ('32) showed that people living at the lower economic levels had the lowest blood vitamin A and carotene while Sie ('37) found that patients with eye symptoms due to vitamin A deficiency also had a low blood vitamin A content. Similar findings were reported by de Haas and Meulemans ('38) who found that children with xerophthalmia had low blood vitamin A and that the blood levels could be increased by vitamin A supplements.

Moore ('39 a, b) and Flora and co-workers ('39) have made determinations of carotene in the blood of dairy cows and calves fed various levels of this substance including the use of carotene from several sources but these investigators have not reported work on the vitamin A content of blood. Studies have been reported by Madsen and Davis ('38, '39) on the spectrophotometric examination of cattle blood for both carotene and vitamin A with the view of using the procedure in the establishment of the minimum and optimum carotene requirements for various physiological functions and for diagnosis of vitamin A deficiency or degree of depletion in cattle. In these studies it was shown that determinations of carotene and relative vitamin A values could be made on extracts prepared from blood plasma. The present paper reports additional studies on methods used and the relation of carotene intake to the carotene and vitamin A content of cattle blood plasma, the relation of the carotene content to the vitamin A content of plasma, together with initial plasma values and observations on the reproductive performance of cows at restricted levels of carotene intake.

EXPERIMENTAL

Preparation of blood plasma sample

Venous blood was drawn from the jugular vein in the morning before feeding. Lithium oxalate (1.5 to 2 mg. per milliliter of blood) was used as the anticoagulant and plasma was separated by centrifuging. Ten to 50 ml. of plasma or serum are required for satisfactory spectrographic determinations of

carotene and vitamin A, depending on the amount present in the sample. However, satisfactory carotene determinations may be made on smaller quantities of plasma from cows receiving adequate carotene. In the use of a 50 ml. sample, the plasma was pipetted into a mixture of freshly prepared alcoholic potassium hydroxide solution made up in the proportions of 75 ml. aldehyde-free absolute ethyl alcohol and 25 ml. of 25% potassium hydroxide. In smaller samples of plasma the amount of alcoholic potassium hydroxide used may be proportionally less. The mixture was then heated on a steam bath in a stream of carbon dioxide for 30 minutes. The digest was cooled and washed into a separatory funnel and extracted by shaking with successive portions of purified [2] petroleum ether (B.P. 35–45°C.). Three or four extractions were usually sufficient. For the large samples 50 ml. of ether for the first extraction and 15 to 25 ml. portions for succeeding extractions were used. The combined ether extracts were washed with water and twice with dilute potassium hydroxide. The water and water-potassium hydroxide layers were discarded when the separation was complete. The extract was then finally washed with water until free from turbidity and potassium hydroxide. The preparation of samples for vitamin A and carotene determinations is the same to this point. Aliquot portions of extract were taken for carotene and vitamin A determinations or separate portions of plasma were extracted for each.

In preparing the extract or an aliquot portion of it for carotene analysis it was further washed with four 25 ml. portions of 92% methyl alcohol after the principle of the Willstätter and Stoll procedure for carotene, dried over anhydrous sodium sulfate, filtered and evaporated to near dryness under reduced pressure in an atmosphere of carbon dioxide. The residue was made up to volume (depending on the concentra-

[2] The ether is treated several times with concentrated sulfuric acid until there is practically no change in color of the acid after standing in contact with the ether for several hours. The ether is then washed with water, treated with potassium permanganate crystals and dried with anhydrous calcium chloride and then distilled through a fractionating column.

tion of carotene) in a mixture of equal parts higher boiling (90 to 98°C.) purified petroleum ether and aldehyde-free absolute ethyl alcohol.

The carotene concentration of the extract was then estimated in a Duboscq colorimeter supplied with a type H4, 100 watt high intensity mercury vapor lamp in connection with a blue filter of high transmission in the region of 4500 Å and using 0.1% potassium dichromate solution as a standard. From the colorimeter readings, sector settings and cell depth for the spectrophotometric examination of the sample were estimated from a curve which was obtained by plotting densities of β carotene solutions determined spectrophotometrically as ordinates against colorimeter readings as abscissas.

Colorimeter readings give only maximum values since no information is given concerning the shape of the absorption curve. Spectrophotometric examination provides data by which the ratios between extinction coefficients at different wave lengths are calculated from which the amount and purity of the carotene in the sample are estimated. A large Littrow spectrograph with a sector photometer attachment is used for this work in this laboratory.

The light source used for the spectrophotometric determination of carotene is a 6 to 8 volt, 32-32 candle-power, automobile headlight bulb, carrying a current of 8.5 amperes. It has been found that this light source is superior to the high-speed steel electrodes used in previous studies because it yields a continuous spectrum in the region where the steel electrode spectrum has only a few lines. The headlight bulb has the additional advantage of running silently during the exposure.

The portion of ether extract of blood plasma used for the determination of vitamin A was dried over anhydrous sodium sulfate after washing with dilute potassium hydroxide and water as previously described, then filtered and evaporated to a small volume (5 to 10 ml.) under reduced pressure in a stream of carbon dioxide. The extract was then transferred to a 25 ml. glass-stoppered flask for final evaporation of the solvent using the same precautions to reduce oxidation of the

vitamin A. Ten milliliters of absolute methyl alcohol was added to the residue. The mixture was shaken and placed in a bath of solid carbon dioxide overnight. Material which precipitated out on cooling was filtered off in a cooled sintered glass funnel and the absorption characteristics of the filtrate were determined spectrophotometrically. Material which was soluble in the cold methyl alcohol and had an absorption maximum at 3280 Å was taken as a measure of the vitamin A in the extract. This method does not give an absolute value for vitamin A since there are other compounds in the extract which affect the nature of the absorption in the vitamin A region. However, such interfering substances do not apparently preclude comparative results.

The source of light for the spectrophotometric determination of vitamin A used in these studies has been either a spark between high-speed steel electrodes or a low-voltage water-cooled hydrogen arc made with a quartz window as described by Allen and Franklin ('39). Curves showing the nature of the absorption spectrum of carotene and vitamin A containing extracts of blood prepared by these methods have been published by Madsen and Davis ('38).

As a general precaution in this work it is desirable to use all glass apparatus with ground-glass stoppers and stopcocks since considerable material may be dissolved from rubber tubing, either cork or rubber stoppers, and some kinds of stopcock grease which interferes with the determinations of vitamin A by the spectrographic procedure. Special care was taken to purify all reagents used and to carry the determinations through as rapidly as possible.

Cattle experiments

Hereford and Shorthorn heifer calves, born at pasture in the spring of 1937, were taken from pasture in the fall, when 6 to 8 months of age, and maintained on a low-carotenoid ration. The constituents of the ration were beet-pulp, white corn, linseed meal, soybean meal, oat straw, salt, and bone-meal, with viosterol in winter. A limited amount of alfalfa hay

was fed for about 2 months until the animals ate the grain ration readily. All but one of these animals were purchased so only approximate ages can be given. After the alfalfa hay was discontinued all the animals developed early symptoms of vitamin A deficiency as determined by ophthalmoscopic examination of the eyes and clinical symptoms such as diarrhea, refusing food, excessive lacrimation, and nightblindness within 3 to 4 months. When symptoms were evident, the animals were given alfalfa leaf meal of known carotene content at several levels of carotene intake, furnishing 30, 45, 60, and 120 micrograms of carotene daily per kilogram of body weight. Blood plasma samples were drawn from these heifers when they were showing symptoms of vitamin A deficiency. Additional plasma analyses were made after the heifers had already reached their maximum concentration of carotene and vitamin A after several months on their respective levels of carotene intake. Blood plasma samples were also analyzed from beef cattle on pasture for comparison with plasma analyses from the animals on restricted levels of carotene intake.

As soon as possible after the heifers had been on their respective levels of carotene intake for a period of 6 months with a previous history of vitamin A depletion, they were bred by a normal bull. Blood plasma samples were again analyzed from the pregnant heifers several days before the calves were born.

RESULTS

Heifers depleted on the low-carotenoid ration and showing early symptoms of vitamin A deficiency all had very low plasma carotene and vitamin A values, while animals on the 30, 45, 60, and 120 μg. of carotene per kilogram of body weight had blood plasma values that were roughly proportional to the carotene intake as is shown in table 1. Determinations made on the blood plasma of normal Shorthorn cows on pasture showed that they had considerably higher carotene and vitamin A values but the vitamin A content of their blood tended to reach a maximum and did not increase proportionally to the carotene concentration. This is illustrated with

additional data in figure 1. The plotted points to which the curve is fitted represent average values showing the relationship of carotene to vitamin A in cattle blood plasma for a total of 107 determinations from forty cattle made on plasma from vitamin A deficient animals, the same animals on the controlled

TABLE 1

Relation of carotene intake to the level of carotene and vitamin A in cattle blood plasma

CAROTENE INTAKE IN µG./KG.	NUMBER OF		CAROTENE IN µG./100 ML.		VITAMIN A IN µG./100 ML.	
	Animals	Analyses	Range	Average	Range	Average
Low[1]	6	14	6–24	14	3–16	10
30	2	8	34–58	46	14–21	18
45	1	4	36–43	41	16–23	19
60	2	5	62–96	81	20–31	26
120	1	2	96–110	103	33–36	35
High[2]	11	11	560–1362	813	40–65	49

[1] Animals depleted on low-carotenoid diet and showing symptoms of vitamin A deficiency.

[2] Animals on pasture.

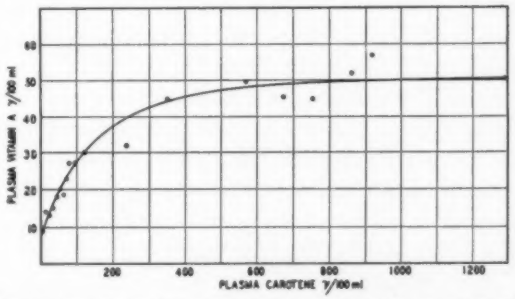

Fig. 1 Micrograms of carotene and vitamin A per 100 ml. cattle blood plasma. A composite curve from analyses of plasma from forty beef cattle including animals with vitamin A deficiency symptoms, animals on restricted levels of carotene intake and animals on normal rations including pasture feeding.

levels of carotene intake and others on normal rations including pasture in some cases.

Considerable variation is evident among the determinations made on the animals on the low-carotenoid ration. This is due in part to various stages of depletion of the carotene and

vitamin A reserves in these animals. Occasional animals had higher vitamin A values than were expected from the carotene content of the plasma and this was interpreted as indicating that the vitamin A reserve was still fairly adequate. However, when the animals continued with low blood carotene, deficiency symptoms developed with further reduction in blood plasma vitamin A.

Variation in the carotene and vitamin A content of the blood is also evident in animals on restricted levels and high levels of carotene intake. For example, two heifers received 30 and 60 μg. of carotene per kilogram of body weight daily during a period of more than 1 year. On the 30 μg. level the plasma carotene varied from 30 to 38 μg. per 100 ml. for heifer 173, and 48 to 58 μg. per 100 ml. for heifer 29. The corresponding range of vitamin A values on these two animals was 10 to 14 μg., and 19 to 21 μg. per 100 ml., respectively. Heifer 166 on the 60 μg. level of carotene varied in plasma carotene from 62 to 78 μg. and in vitamin A from 20 to 29 μg. per 100 ml., while heifer 98 received the same amount of carotene per unit of body weight and varied in plasma carotene from 84 to 96 μg. and in vitamin A from 23 to 31 μg. per 100 ml.

The reproductive performance and the carotene and vitamin A values of the blood plasma for the pregnant heifers shortly before calving are given in table 2. These plasma values are not included in table 1.

Heifers receiving 30 and 45 μg. of carotene per kilogram of body weight produced calves that were either blind and weak, or dead at birth. The calf from the heifer 78 on the 45 μg. level was stronger at birth than the calf from heifer 173 on the 30 μg. level and grew apparently normally but began to develop typical vitamin A deficiency convulsions at 21 days of age and died 23 days later. Both of the calves born to the animals on the 60 μg. level were apparently normal at birth. Heifer 98 had 96 μg. of carotene and 22 μg. vitamin A per 100 ml. plasma and her calf gained well and was still apparently normal at about 4 months of age. The other calf whose mother had less plasma carotene but with the same plasma vitamin A was

normal until about 1 month of age at which time he began to have occasional convulsions. Supplements of magnesium citrate, additional viosterol and carotene failed to correct the condition and the animal died about 2 months later.

Heifer 234 on the 120 μg. level of carotene, in contrast to the two heifers receiving only half as much carotene, aborted about the eighth month of pregnancy. A post mortem examination of this heifer was made 4 days after aborting and infantile ovaries and a cystic pituitary gland were observed. The plasma carotene and vitamin A of this animal were indicative of her higher level of carotene intake. The control animal,

TABLE 2

Reproductive performance, blood plasma carotene and vitamin A of individual pregnant heifers 1 to 7 days before calving when fed controlled levels of carotene

HEIFER NO.	CAROTENE INTAKE OF HEIFER	OBSERVED CONDITION OF CALF	BLOOD PLASMA	
			Carotene	Vitamin A
	μg./kg.		*μg./100 ml.*	*μg./100 ml.*
173	30	Weak; blind; convulsions	30	10
29	30	Died at birth	42	14
78	45	Blind; convulsions in 3 weeks	59	20
98	60	Very good	96	22
166	60	Good	78	22
234	120	Dead; premature birth	116[2]	24[2]
169	control[1]	Excellent	143	24

[1] Received 2 kg. U. S. No. 1 alfalfa hay daily.

[2] Plasma sample taken day after abortion.

heifer 169, with a slightly higher plasma carotene than the heifer receiving the 120 μg. level of carotene, but with the same concentration of plasma vitamin A, had a strong normal calf.

DISCUSSION

Analysis of cattle blood plasma for carotene and vitamin A by the procedure described indicates that long continued inadequate carotene intake and vitamin A deficiency in cattle can be determined by blood analysis. Preliminary findings reported by Mohler ('39), which include some of the data given in the present paper, indicate that the critical level of carotene

in the plasma is about 25 μg. per 100 ml. and for vitamin A in the same sample about 16 μg. per 100 ml. Animals having carotene and vitamin A values at these levels or above usually do not show the characteristic clinical symptoms of vitamin A deficiency.

These results with regard to the critical level of carotene are essentially in agreement with those of Moore ('39 b) who reports that plasma carotene at 0.2 μg. per milliliter or above was sufficient to prevent nyctalopia (nightblindness) and to maintain fair general health in Holstein and Ayrshire calves. In the same study, Moore noted that nyctalopia and papillary edema followed when the carotene values fell below about 0.13 μg. per milliliter. It is possible that the difference in the critical level of carotene in the plasma as found in this investigation and those of Moore may be partially accounted for by difference in the breed of cattle studied.

Plasma carotene has been shown to increase to higher levels with increasing carotene intake, but plasma vitamin A, as measured by the method described, tends to reach a less variable maximum concentration at a relatively early point. This may be due to a physiological balance between rate of carotene conversion into vitamin A and vitamin A utilization.

It is evident that much more carotene is needed and consequently higher plasma carotene and vitamin A levels are necessary for heifers to give birth to normal calves than to prevent the development of typical symptoms of vitamin A deficiency. The two heifers receiving 60 μg. of carotene per kilogram body weight from high grade alfalfa leaf meal were able to produce apparently normal calves at birth, but this level of carotene intake cannot be considered a safe level for practical recommendations since one of the calves later developed deficiency symptoms. However, these findings, although representing only a few animals, provide some initial information on the levels of plasma carotene and vitamin A that must be maintained in the pregnant animal in order to expect the birth of normal or deficient calves. Undoubtedly, the carotene intake of cattle which is sufficient to maintain approximately maximum vitamin A values in the plasma is more than suffi-

cient for the prevention of the usual symptoms of vitamin A deficiency and should be optimum for reproduction.

Converse and Meigs ('38) found that when timothy or clover hay was fed without pasture a daily intake of 80 to 100 mg. of carotene seemed sufficient to produce normal calves but that weak or dead calves were expected when the daily intake of carotene was 50 to 60 mg. These values are somewhat higher than those suggested by the limited number of cases studied in the present investigation. These results should not be considered as conflicting since the breed of animals used, source of carotene and other conditions of the experiment are not comparable.

SUMMARY

A method is described for the spectrophotometric determination of carotene and vitamin A in cattle blood plasma. The method is quite satisfactory for the determination of carotene but vitamin A values must be considered relative because of substances in the plasma extract which cause some interfering absorption.

Vitamin A deficiency in cattle or an inadequate intake of carotene resulting in depletion of the animal's reserve can be detected by blood carotene and vitamin A analysis. The carotene and vitamin A content of blood plasma are dependent on the carotene intake and previous storage of these factors. After cattle have been depleted of carotene reserves, the blood carotene level is dependent on the carotene intake although there is some individual variation. The vitamin A content of blood plasma is closely related to its carotene content. However, vitamin A values tend to reach a stable level and do not increase proportionally with increasing carotene intake.

Apparently normal calves were born in two cases to beef heifers that received 60 μg. of carotene from high quality dehydrated alfalfa leaf meal per kilogram of body weight previous to and throughout the gestation period. However, one of the calves gave signs of being deficient at about 1 month of age. Heifers receiving 30 and 45 μg. of carotene produced deficient calves although the cows themselves remained apparently normal.

Blood plasma carotene and vitamin A values are given for a wide range of carotene intake and for seven heifers which gave birth to deficient or normal calves during the experiment.

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EFFECT OF A.T.10 (DIHYDROTACHYSTEROL) ON RICKETS IN RATS PRODUCED BY HIGH-CALCIUM-LOW-PHOSPHORUS DIETS

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ONE FIGURE

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A.T.10, an irradiation product of ergosterol, is known to have important effects upon calcium and phosphorus metabolism. This has been amply demonstrated not only by Holtz et al. ('34), its discoverers, who showed its ability to raise serum calcium concentration after removal of the parathyroids, but also by the studies of Albright and collaborators ('38, '39) on the metabolism of calcium and phosphorus in idiopathic hypoparathyroidism and in rickets. They have compared the effects of A.T.10 to those of vitamin D_2 and parathyroid extract. It is their thesis that vitamin D has two primary actions, to increase calcium absorption from the gastrointestinal tract and to increase phosphate excretion in the urine, whereas parathyroid hormone does not increase calcium absorption but does increase the phosphate in the urine markedly. According to these authors A.T.10 has the same two actions as vitamin D, but increases calcium absorption less and phosphate excretion more. In confirmation of this hypothesis it has been shown that A.T.10 will prevent or cure rickets in rats produced by a low-calcium-high-phosphorus diet in the same dosage that is without effect on the rickets produced by classical high-calcium-low-phosphorus diets (Shohl, Fan and Farber, '39). It has been reported by

Harnapp ('35) that A.T.10 has 1/400–1/600 the antiricketic potency of vitamin D_2. This has been confirmed.[1]

The present study was made to test whether A.T.10 in any amount is incapable of curing rickets produced by high-calcium-low-phosphorus diets and if so, if the healing is identical with that produced by vitamin D. In other words, does it resemble parathyroid hormone, which intensifies the ricketic process or delays healing, or does it resemble vitamin D in its action?

Like both parathyroid hormone and vitamin D, A.T.10, when given in sufficient amounts, has toxic effects. In fact, this material has been standardized on the basis of "borderline toxic dose" (in this paper called "minimal toxic dose") which involves the loss of weight or death of mice. We have studied the quantitative relationship between the amounts of A.T.10 which produce therapeutic and toxic effects.

EXPERIMENTAL PROCEDURE

Young albino rats of 28 to 30 days of age and 48 to 53 gm. in weight [2] were divided at random into six groups of four each and placed upon the high-calcium-low phosphorus ricketogenic diet no. 2965 of Steenbock and Black (76% fresh ground whole yellow corn, 20% gluten flour, 3% $CaCO_3$, and 1% NaCl). The rats of group 1 served as rickets controls (negative controls); those of group 2 were fed protective doses of cod liver oil containing 7 International Units of vitamin D per rat per day (positive controls); those of groups 3, 4, 5, and 6 were given 5, 25, 125 and 375 μg. of A.T.10 [3] respectively, as shown in table 1. The rats which received the "normal" diet (Sherman diet B) were not litter mate controls; they were used only to show the order of magnitude of findings in normal animals. All supplements were given daily in 1 drop doses, put directly into the rat's mouth. All dilutions of A.T.10 were made with

[1] Unpublished data of Dr. O. W. Barlow of the Winthrop Chemical Co.

[2] Obtained from Sprague-Dawley Co., Madison, Wis.

[3] The A.T.10 used was obtained through the Division of Medical Research of the Winthrop Chemical Company.

sesame oil (which was the same oil used by the manufacturer in making the preparation) in such concentration that the daily dose could be given in a single drop. The animals were weighed weekly.

At the end of 20 days the animals were x-rayed and the next day were killed by ether anesthesia and bled from the femoral vessels. The pooled blood serum of each group was analyzed

TABLE 1

GROUP	DIET AND SUPPLEMENT[1]	BLOOD SERUM			BONES			
		Calcium	Phosphate	Phosphatase	Ash of fat free femur	Ash	X-ray[2]	Histology[2]
		mg./100 cc.	*mg. P/100 cc.*	*Bodansky units*	*%*	*mg.*		
	Normal	10.5	8.2	21.5	56.0	84	0	0
1	Rickets control	10.9	3.3	48.2	37.6	35	++++	++++
2	Cod liver oil, 8 units	12.4	6.2	34.3	48.0	48	0	0
3	A.T.10, 5 μg. (⅕ M.T.D.[3])	11.7	4.8	36.7	46.0	50	+++	++
4	A.T.10, 25 μg. (1 M.T.D.)	12.1	6.8	32.7	45.4	41	0	0
5	A.T.10, 125 μg. (5 M.T.D.)	12.6	8.7	27.3	53.0	56	0 *h*?	0 *h*
6	A.T.10, 375 μg. (15 M.T.D.)	16.0	6.8	24.8	47.5	48	0 *h*	0 *h*

[1] Normal diet was Sherman diet B. Groups 1–6 received diet no. 2965 of Steenbock and Black.

[2] The number of + signs indicates the severity of rickets. 0 means no rickets. *h* means hypercalcification at provisional zone of calcification.

[3] Minimal toxic dose.

for calcium, inorganic phosphate and phosphatase. The femurs of the right legs were dissected out and analyzed for water, fat, other organic matter and ash, by the methods previously described (Shohl et al., '39). The animals were then autopsied and the organs and bones preserved for histologic examination.

The experiment proceeded without known error. Daily observation disclosed little except that the animals which received the smallest doses of A.T.10 seemed more active than the rickets controls, and those which received the largest doses

were more torpid. The animals receiving the largest doses became progressively more emaciated. On the morning of the twenty-first day of the experiment one animal in this group was found moribund, and died before blood could be obtained for analysis.

RESULTS

Weight. The weight curves given in figure 1 demonstrate that 125 and 375 μg. of A.T.10 per rat per day (5 and 25 minimal toxic doses) caused definite loss of weight. In group 5

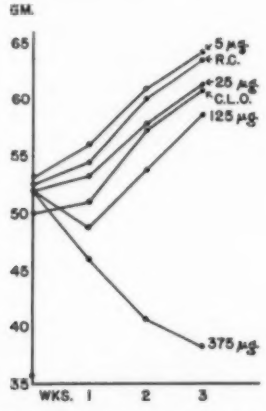

Fig. 1 Growth of rats fed high-calcium-low-phosphorus ricketogenic diet with supplements of A.T.10. R.C. = rickets controls. C.L.O. = cod liver oil supplement, 8 units daily.

this decrease was of only 1 week's duration, but in group 6 the weight loss continued, and one animal died. This must be regarded as definite evidence of toxicity. Further significance of the weight losses is discussed later.

Blood serum. The data are summarized in table 1. The rats of group 3, which received the smallest doses of A.T.10, showed higher calcium and phosphate values than the rickets controls. Each of groups nos. 4 to 6 received more A.T.10 than the one preceding it; correspondingly the serum calcium concentration of each group was higher than the one before it.

Only in group 6 was hypercalcemia marked. Similarly the serum phosphate concentration was greater with larger dosage, and approached normal values. The higher serum phosphate values were accompanied by lower phosphatase concentration.

Judged by these criteria alone the conclusion would be reached that the smallest doses of A.T.10 used (5 μg.) exerted a slight but definite antiricketic effect.

Bone analysis. Determination of the bone ash, which is probably the most sensitive method of demonstrating changes in calcium metabolism, shows the usual values for rickets controls and for the rats protected by cod liver oil (table 1). In all groups fed A.T.10 advanced mineralization took place. Even with the toxic doses the bones were not small in size, and they did not contain less ash, either absolutely or relatively than those of the rats which received cod liver oil. Judged by these analyses also it is concluded that 5 μg. of A.T.10 produce a definite antiricketic effect.

X-ray and histology. More weight is given to the interpretation of roentgenograms and histologic examination than to blood findings and bone ash (table 1).

In agreement with the roentgenograms, histologic examination of the tissues demonstrated that group 3 showed evidence of slight but definite antiricketic effect. Groups 2 and 4 showed marked healing. Groups 5 and 6 showed no rickets. Group 5 showed moderately dense, and group 6 very dense deposition of calcium mainly at the provisional zone of calcification, and to a lessened degree in the shaft. Thus all evidence is in agreement, except that in group 6 the bone ash is less than in group 5, both relatively and absolutely although hypercalcification is greater. This is interpreted as dissolution of bone salts from the shaft and their deposition at the provisional zone of calcification. The details of the histologic changes in this and related conditions will be described elsewhere. So far as could be determined the mechanism of healing of the ricketic lesion, following administration of A.T.10, was similar in all respects to that resulting from vitamin D.

DISCUSSION

From these data we conclude that, in the dosages used, A.T.10 protected rats from development of rickets produced by a high-calcium-low-phosphorus ricketogenic diet. Presumably the active agent was dihydrotachysterol. Unfortunately this cannot be stated definitely because the only product available is produced under German patents by secret processes abroad. There is no way of determining whether the effect of the preparation may be due to impurities such as other irradiation products of ergosterol or vitamin D itself. For this reason the term A.T.10 has been used rather than dihydrotachysterol.

It is well known that when rats receive food in inadequate amounts so that loss of weight occurs, healing of rickets takes place. Presumably this is due to the phosphorus supplied by the destruction of tissue. Under conditions in which weight losses occur it is therefore impossible to attribute the healing of rickets to the direct action of the drug studied. Therefore it is necessary to exclude from discussion the prevention of rickets in groups 5 and 6.

The factor of safety between the healing dose and the toxic dose of A.T.10 should be noted. It requires 0.0625 μg. of vitamin D to prevent rickets and 625.0 μg. will cause hypervitaminosis; with A.T.10, 25 μg. will afford protection, and 125 μg. will cause toxic symptoms. The toxic dose of vitamin D is approximately 10,000 times the therapeutic dose; with A.T.10 it is only about five times. Stated in other terms, A.T.10 is 1/400 as effective as vitamin D in curing of rickets and is five times as toxic.

The effect of A.T.10 on calcium and phosphorus metabolism is beyond question. In our previous study (Shohl et al., '39) with rickets produced by a low-calcium-high-phosphorus diet, healing resulted from an amount of A.T.10 approximately 1/80 the amount that was effective in this experiment. Thus the effect on calcium metabolism is demonstrable, under suitable conditions, with amounts which are much smaller than those which cause toxic manifestations. However, the highest

dosage used in the previous experiment (3 μg.) did not cause healing of rickets produced by a high-calcium-low-phosphorus diet. The smallest dose used in this experiment is approximately twice the largest dose used previously; the previous conclusions are therefore confirmed and extended.

In the previous experiment no increase in serum calcium concentration was found; the amounts of A.T.10 used in this experiment definitely raised serum calcium levels. Here again the property of increasing serum calcium concentration is found only when the dosage is increased to the point near which toxic symptoms appear.

Deposition of minerals may occur at the epiphysis when bone salts are being dissolved from the shaft and deposited at the provisional zone of calcification. This might take place without increase in the calcium absorption. Evidence of increased absorption is furnished when the balances of calcium and phosphorus are positive. However, equally good evidence of increased absorption is given in this case by the increase in the absolute amount of ash per femur. This can be present only if the balances are positive.

A.T.10 resembles vitamin D and differs from parathyroid hormone because it increases absorption of calcium, as shown by the fact that the bones of animals given A.T.10 increased in minerals both absolutely and relatively, compared to the rickets controls. If the principal effect of A.T.10 were to increase calcium absorption this would be most readily detectable when the calcium intake was high, but we previously showed that it was more effective in the cure of rickets when the phosphorus intake was high and the calcium low. Therefore, A.T.10 exerts its effect principally on the excretion of phosphate in the urine; and to a lesser extent on the absorption of calcium. This is in agreement with the hypothesis presented by Albright et al. ('38, '39).

Histologic examination, although it cannot differentiate between this type of healing and that produced by vitamin D, shows clearly that in this case, as well as with low-calcium diets, the prevention of rickets with A.T.10 can be accom-

plished without toxic manifestations. This indicates further that whatever may be the mechanism of deposition of minerals in uncalcified cartilage, in these and similar conditions it is primarily the calcium and phosphorus metabolism that determines whether rickets is present or not.

SUMMARY

A.T.10 in amounts which approximate toxic dosage, is effective in prevention of experimental rickets in rats fed a high-calcium-low-phosphorus ricketogenic diet.

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THE CALCIUM CONTENT OF THE NORMAL GROWING BODY AT A GIVEN AGE

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THE PROBLEM

An outstanding feature of the chemistry of nutrition is the fact that the body, in its normal growth and development, must increase not only the amount but also the percentage of calcium which it contains. The purpose of the present work is to ascertain whether this normal increase in the percentage of calcium in the growing body is inherently related to age, or is significantly influenced by differences in growth rate within the normal zone.

Liebig taught the view that the individual plant or animal grows only at the rate or to the extent consistent with the specific chemical composition of its kind. From this it would follow that the rate of growth would be set by any substance or element essential to the body composition which is present in minimal proportion relative to need for growth at a normal rate. Hence this idea has often been called Liebig's "Law of the Minimum." Now after nearly a century, we can see that some aspects of growth and development do "obey" this so-called law while other aspects do not. Thus among the many published investigations of the effects of shortages of specific amino acids we find no report of animals growing-up with, for instance, lysine-poor bodies; while there is no lack of evidence that shortage of calcium in the food may result in a calcium-poor body.

Aron and Sebauer ('08) published observations upon dogs of essentially the same age and size, one of which had grown

up on normal, the other on calcium-poor food. The fresh bones of the normal and calcium-poor animals of the same size weighed about the same; but upon drying, those of the calcium-poor dog were found to contain more water and less calcium than those of dogs receiving food of normal calcium content.

Osborne and Mendel ('18) showed that some growing animals on a calcium-poor diet were promptly checked in growth while others grew some time before showing any outwardly visible effects of calcium deficiency.

Sherman and Hawley ('22) in work with children found that those with the higher calcium intake stored more and thus must have had a higher calcium content at a given age.

Moulton ('23) studied the composition of the ox and man from early embryonic stage to maturity. The water content of these mammals decreased very rapidly from conception to birth, and then less rapidly until in each case a stable composition was reached. This condition, of a stabilized or plateaued chemical composition of body, Moulton called "chemical maturity"; this term thus referring to the percentage and not to the amount of the given chemical constituent in the body.

Sherman and MacLeod ('25) found the body calcium of normal rats to increase from about 0.25% at birth to about 1.25% at maturity. Even in this long-controlled, laboratory-bred colony there is (as in a human population) considerable individual variability in the rate of growth.

The problem of the investigation reported in this paper was therefore to find whether in the normal animal it is primarily the age or the individual rate of growth which determines the percentage of calcium in the body; or whether progress toward "chemical maturity" in Moulton's sense is (in the case of calcium) determined essentially by age or by growth. This problem has been given increased importance by the reference of Shohl ('39) to the change in the body's composition as correlated with its increase in size, though the context does not indicate that discrimination between age and size was definitely intended.

EXPERIMENTAL

The experimental animals were albino rats of Osborne-Mendel stock which during its period of about fifty generations in the Columbia laboratory has been bred from average members of each successive generation and so has not increased so much in individual size as have the rats of the many colonies in which the tendency is to select animals of superior size for breeding.

In the present work, all the members of a given litter were killed for analysis at the age of 28 days, which in terms of human analogy may be considered as the end of infancy. This was the age at which largest numbers of animals were available for our analyses, and it is well within the period of rapid growth in which a distinction between age and size should have its greatest significance. Each sex was considered as constituting a separate series; and as animals from families on three different diets were treated separately, there were in all six series of analyses each of animals homogeneous except for individual differences in growth and resulting size at a given age.

We wish to make grateful acknowledgment to Dr. H. L. Campbell of this laboratory, under whose direction the animals were reared.

The three dietaries or family nutritional backgrounds were: (1) diet 13, consisting of two-thirds ground whole wheat and one-third dried whole milk, and containing 0.35% of calcium in the dry food mixture; (2) diet 132, differing from diet 13 in that enough calcium carbonate was added to bring the calcium content of the dry food to 0.49%; and (3) diet 133 in which enough more calcium carbonate was added to bring the calcium to 0.64% of the dry weight of the food. These food-mixtures, each of which contained from 0.49 to 0.50% of phosphorus and from 16.1 to 16.2% of protein, were fed with sodium chloride and distilled water.

The results of the analyses of the animals of each of the six series (each sex from each of the three family diets) were studied statistically, for possible influence of body size at the given age, in two ways: first, by averaging in three groups (table 1) the "large", "medium", and "small" members of each litter, with about half of the cases assigned to the medium

TABLE 1

Calcium contents of 28-day-old rats grouped according to size as the small, medium, and large members of their respective litters

SEX AND NUTRITIONAL BACKGROUND	SIZE	NO. CASES	NET BODY WEIGHT	BODY CALCIUM	
			gm.	*gm.*	% and P.E.[1]
M. Diet 13	Small	16	40.4	0.299	0.74 ± 0.005
	Medium	31	46.0	0.336	0.73 ± 0.004
	Large	16	55.5	0.396	0.71 ± 0.003
M. Diet 132	Small	16	37.1	0.305	0.82 ± 0.008
	Medium	32	43.9	0.350	0.80 ± 0.004
	Large	16	53.2	0.422	0.79 ± 0.004
M. Diet 133	Small	18	37.5	0.326	0.87 ± 0.005
	Medium	36	47.3	0.406	0.86 ± 0.005
	Large	17	54.7	0.446	0.82 ± 0.006
F. Diet 13	Small	18	38.2	0.295	0.77 ± 0.004
	Medium	33	43.5	0.326	0.75 ± 0.004
	Large	19	49.9	0.370	0.74 ± 0.005
F. Diet 132	Small	20	33.5	0.282	0.84 ± 0.006
	Medium	34	41.4	0.338	0.82 ± 0.004
	Large	18	48.6	0.400	0.82 ± 0.004
F. Diet 133	Small	20	35.1	0.321	0.91 ± 0.006
	Medium	40	44.4	0.397	0.89 ± 0.005
	Large	20	52.2	0.444	0.85 ± 0.006

[1] P.E. = probable error of the mean, as fully explained by Chaddock ('25).

group; and second, by averaging in two groups consisting respectively of those above and those below the mean weight of their litter and designated (in table 2) as "heavier" and "lighter". The animals were prepared and cremated, and their calcium contents determined by the McCrudden method, with the precautions described by Sherman and MacLeod ('25).

DISCUSSION

For economy of space the coefficients of variation are not tabulated, since they are all of the order of 3 to 5 with no consistent indication that either the smaller or the larger animals were more variable than the average of their series. Hence, also, variability was satisfactorily low in all groups and the conclusion to be drawn as to the relative influence of age and size is strengthened. As the direct relation of body calcium to age was established by the work of Sherman and MacLeod ('25), the problem here becomes whether or not the percentage at a given age is measurably influenced by differences (within the normal range) in the rate at which the individual has grown as shown in the weight of the body at a given age.

All six of the comparisons summarized in table 1 show slightly higher percentages of calcium in the "small", and five of the six show slightly lower percentages in the "large", than in the "medium" or average of their respective age, sex, and nutritional background. All these differences are, however, so small that none of them considered independently would be regarded as statistically significant.

For further statistical scrutiny, the animals of each of the six series were divided again according to size but into only two groups: the "heavier" and "lighter" halves. The numerical findings as summarized in table 2 show higher mean percentages of calcium in the lighter animals in all six of the comparisons. The differences, however, appear here as in the previous mode of interpretation to be insignificant compared with such differences as have been found due to age or to level of calcium intake. If the ratio of each difference to its probable error be taken alone and given the conventional interpretation, half of them would on the face of the figures appear "significant" and half "insignificant"; but in the light of all the evidence, and particularly of the fact that all the differences are near the limit of accuracy of the best experimental and analytical methods, it does not seem best in this case to say that some of the differences are significant and others are

not. Neither a separate comparison according to sex nor a separate comparison according to nutritional background shows any consistent trend of difference. There is probably a very slight inverse relationship between the size of the body and its percentage of calcium at the given age here studied; but this, if it exists, is so small a factor as to be only doubtfully measurable, and practically insignificant as compared with the age factor.

TABLE 2

Calcium contents of 28-day-old rats grouped according to size as the "lighter" and "heavier" halves of the individuals of their respective series

SEX AND NUTRITIONAL BACKGROUND	SIZE	NO. CASES	NET BODY WEIGHT	BODY CALCIUM	
			gm.	*gm.*	% and P.E.[1]
M. Diet 13	Lighter	31	42.2	0.310	0.74 ± 0.004
	Heavier	32	51.6	0.372	0.72 ± 0.003
M. Diet 132	Lighter	32	39.6	0.321	0.81 ± 0.005
	Heavier	32	49.5	0.393	0.79 ± 0.004
M. Diet 133	Lighter	36	41.3	0.360	0.87 ± 0.004
	Heavier	35	52.0	0.431	0.83 ± 0.005
F. Diet 13	Lighter	36	40.0	0.306	0.76 ± 0.004
	Heavier	34	47.9	0.356	0.74 ± 0.004
F. Diet 132	Lighter	37	36.5	0.303	0.83 ± 0.004
	Heavier	35	45.8	0.376	0.82 ± 0.003
F. Diet 133	Lighter	38	38.6	0.352	0.91 ± 0.004
	Heavier	42	48.9	0.423	0.87 ± 0.004

[1] P.E. = the probable error of the mean.

Hence whichever the mode of comparison and interpretation of the data, it appears that the percentage of body calcium in the normal growing animals of the same sex and nutritional background, was essentially determined by their age, the smaller animals containing only a very slightly higher percentage of calcium than the larger animals of the same age and sex.

As all of the foregoing discussion is in terms of percentages of body calcium, it obviously follows that the amount of calcium in the body was larger in the larger rats and smaller in the smaller rats, almost in proportion to their body weight.

SUMMARY AND CONCLUSION

Normal animals at a given age were compared (males and females separately) as to whether the percentage of body calcium differed materially with the body weight of the animal; i.e., with the rate at which it had grown. Such comparisons were made independently with animals from three different diets representing successive levels of liberality of calcium intake. Thus there were six comparisons, each embracing from sixty-four to eighty animals.

In none of these six comparisons did the larger animals show a higher percentage of body calcium than the smaller animals of the same age, as would be the case if the higher body weight meant greater maturity of skeletal development at the age studied (28 days in the rat, or "end of infancy").

Obviously the amount (total weight) of calcium in the body tends to vary with its size. The comparisons which follow are therefore made on the basis of percentage of body calcium.

In all of the six comparisons, the smaller animals show a slightly higher, and the larger animals a slightly lower, percentage of body calcium than the general average of their group. The differences, however, were only such as might be regarded as being near the limit of accuracy of the method of experiment and analysis, and so on the border line of possible statistical significance.

Thus the rate of growth as expressed by the body weight at a given age, is in itself not a disturbing factor in the rate of calcification involved in normal development. This finding is of special significance in the interpretation of certain investigations upon the influence of food.

These analyses of well over 400 experimental animals establish the fact that among normal growing individuals of a given sex, having the same hereditary and nutritional background, age is the predominant determining factor in the increasing percentage of calcium in the body.

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A QUANTITATIVE STUDY OF VITAMINS IN THE RUMEN CONTENT OF SHEEP AND COWS FED VITAMIN-LOW DIETS

III. THIAMIN

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ONE FIGURE

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Early investigations (Theiler et al., '15; Bechdel et al., '26, '27, '28) dealing with the vitamin B requirements of ruminants were handicapped by the existing lack of knowledge regarding the complex nature of the B vitamins and by the lack of well-defined methods of assay. Reinvestigation of the problem in the light of more recent knowledge is therefore timely. It was previously reported (McElroy and Goss, '39) that thiamin was synthesized in the rumen of sheep. The animals were fed a ration deficient in thiamin and their rumen and reticulum contents were found to contain more than 5 μg. of thiamin per gram. Additional assays have been made to determine more accurately the thiamin potency of this sheep rumen and reticulum material and also to determine if a similar synthesis of thiamin occurs in the rumen of the cow.

EXPERIMENTAL

The ruminant ration and the method used to obtain samples of rumen contents from the experimental animals were the same as described in previous papers (McElroy and Goss, '40 a, '40 b).

Thiamin assays of sheep rumen contents. Thiamin assays were conducted by the method of Jukes and Heitman ('40), in which the degree of postponement of fatal polyneuritic symptoms afforded by the test material is compared with that afforded by graded levels of synthetic thiamin chloride hydrochloride [1] added to chick diet 152, deficient in thiamin.

In the first thiamin assay the dried sheep rumen and reticulum contents were fed at a level of 20%. Shortly after the

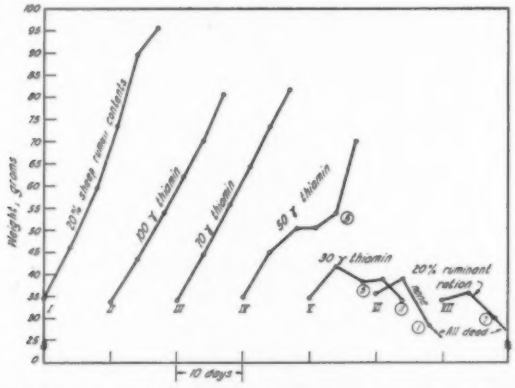

Fig. 1 Growth curves of chicks in the first thiamin assay of ruminant ration and of dried rumen and reticulum contents. Twelve chicks were included in each group. The figures in circles under the curves represent the cumulative number of deaths from polyneuritis. The last part of the growth curve for groups VI and VII is shown as a broken line since no weights were taken during this interval. Supplements added to basal diet 152 are shown above the curves. The amounts of thiamin indicated were added per 100 gm. of diet.

beginning of the trial it became evident that the thiamin potency of the test material had been underestimated and the assay was stopped on the seventeenth day since it had become obvious that the rumen material contained over 5 μg. of thiamin per gram and it would be necessary to feed it at a lower level in order to obtain an assay. The growth made by the different groups up to the seventeenth day is shown in figure 1. When the trial was stopped the chicks in groups I

[1] Merck and Company.

and II were changed to the basal diet and further evidence for the high thiamin content of the sheep rumen and reticulum material was supplied by the fact that the average postponement of fatal polyneuritis in the group fed sheep rumen and reticulum contents was 13.1 days as compared to 8.3 days for the group which had previously received 100 μg. of thiamin per 100 gm. of diet. Jukes and Heitman ('40) have estimated that diet 152 contains less than 0.4 μg. of thiamin per gram. The results for groups VI and VII indicate that the ruminant ration did not contain appreciably any more thiamin than diet 152 since supplementing this diet with 20% ruminant ration failed to delay the onset of fatal polyneuritis.

TABLE 1

Thiamin assay of ruminant ration and sheep rumen contents

GROUP NO.	AMOUNT OF SUPPLEMENT ADDED TO BASAL DIET 152	NUMBER OF CHICKS AT BEGINNING OF TRIAL	GROUP POLYNEURITIC MORTALITY INDEX [1]
I	0	12	19.0
II	5% ruminant ration	12	18.8
III	5% sheep rumen and reticulum contents	12	14.9
IV	50 μg. thiamin per 100 gm.	12	9.4
V	70 μg. thiamin per 100 gm.	12	5.6

[1] The "polyneuritic mortality index" was calculated for each chick by subtracting the number of days it survived from the number of days (28) in the assay period. Complete protection was therefore indicated by an index value of zero.

The results of the second thiamin assay of sheep rumen and reticulum contents in which the test material was fed at a level of 5% are summarized in table 1. From the data in table 1 and the response curve of Jukes and Heitman ('40) it was estimated that the sheep rumen and reticulum contents contained approximately 7 μg. of thiamin per gram.

This represents a seventeenfold increase in thiamin potency for the rumen and reticulum contents over the ruminant ration and reasons have been presented in a previous communication (McElroy and Goss, '40 b) for considering increases of this magnitude as evidence of vitamin production,

presumably by microbial growth, rather than of a simple concentration of vitamin already present in the ration.

EXPERIMENTS WITH COWS

In preliminary work a pure bred Jersey cow with a permanent fistula into the rumen was used. Considerable difficulty was encountered in getting this cow to accept the experimental feed without a supplement of a small amount of alfalfa hay and when, after 5 weeks, hay was omitted entirely from the ration, severe anorexia developed after 8 days and the cow refused to eat any of the experimental feed. At this time a sample of rumen contents was removed for drying and subsequent biological assay and the cow was turned out to pasture to recover. However, neither her appetite nor rumen activity was restored and 4 days later she died with symptoms suggestive of thiamin deficiency paralysis. A post mortem examination failed to reveal any acute condition other than putrefaction of the rumen contents. No thiamin could be detected by biological assay with chicks in the sample of rumen contents removed before the cow was turned out to pasture. These results suggested the possibility that thiamin deficiency may have been the primary cause of complete failure of appetite and subsequent death of this animal.

Since, in the experiments with sheep described above, no cases of anorexia developed and subsequent assays indicated the synthesis of an appreciable amount of thiamin in the intact rumen of this species, results of the opposite nature with the bovine had not been anticipated. One possible explanation of the difference appeared to be that the presence of a fistula into the rumen of the cow might have had an inhibitory action on the growth of rumen organisms capable of synthesizing thiamin, and this combined with loss of rumen fluid through leakage might have contributed to a severe thiamin deficiency. Therefore, in order to test the possibility that a fistulated animal might not be a satisfactory subject for this type of experiment, one normal or non-fistulated cow,

no. 706, and one fistulated cow, no. 557, were used in subsequent studies.

Both of these cows accepted the experimental ration much more readily than did the cow used in the preliminary trial. Nevertheless anorexia developed at intervals during the course of the experiment. The normal cow, no. 706, went off feed twice during the 3 months she was fed the vitamin deficient ration, but both times regained her appetite after starving for 18 to 36 hours. The fistulated cow, no. 557, refused to eat for periods of 24 to 56 hours six times during the 5 months of experimental regime.

In view of the previous experience which suggested that the cause of anorexia might be thiamin deficiency, intravenous thiamin injections were tried on the three occasions when no. 557 refused to eat for more than 36 hours. Successively larger doses of 50, 100 and 500 mg. of thiamin were used. The results were indefinite. When the 50 mg. injection was given the cow refused to eat 1 hour after the injection, but accepted the feed after 6 hours. It was thought that perhaps a larger dose would produce a more rapid and marked desire to eat, but when the dose was increased to 100 and 500 mg. on succeeding occasions the cow failed to eat until 8 and 14 hours respectively after the injection was made. The possibility that the cow would have accepted the experimental feed just as soon without having been injected with thiamin cannot be eliminated. Nevertheless the following observations may be significant:

1. The rumen was either quiescent or very sluggish before the injections were made but became active within 1 to 3 hours after injection.

2. In the case of the first and second injections the cow was noted to lick the empty feed trough and exhibit signs of hunger after 3 to 4 hours, even though she still refused to eat when feed was placed before her.

3. Before injection the muzzle was dry and there was a thick discharge from the nostrils. The muzzle always became

moist within a few hours after the injection and the nasal discharge disappeared within 24 to 72 hours.

Samples of rumen contents were taken from this cow only when her appetite was good and, as will be shown later, no thiamin was detected in these samples.

With regard to the non-fistulated cow used as a control, we were not successful in obtaining a satisfactory sample of her rumen contents for biological assay. After this cow had been on the experimental diet for 88 days she was slaughtered for the purpose of obtaining her rumen contents but unfortunately the bulk of the rumen material was lost during the slaughtering operation. Only a small amount of material retained in the folds of the rumen was saved and as this contained a considerable proportion of sand and small bits of gravel it was by no means a representative sample of the organic matter present in the rumen of the animal during the experimental period.

Thiamin assays of cow ration, rumen contents, and skim milk from cow no. 557. As mentioned in an earlier paper (McElroy and Goss, '40 a) fistulated cow no. 557 calved during the course of the experiment and consequently samples of both her rumen contents and milk were available for thiamin assay. In preparation for thiamin assay the milk was first skimmed and then concentrated 4.7:1 under reduced pressure at a maximum temperature of 38°C. This concentrate was subsequently mixed with a known weight of dry basal diet 152 and dried to a constant weight in a current of air at 40°C. after which sufficient diet 152 was added to lower the concentration of dried skim milk to 15% or 23% as desired. A sample of commercial skim milk powder prepared by the drum process was assayed at the same time. The results of the assay are summarized in table 2.

Table 2 shows that no thiamin was detected in the ruminant ration by biological assay with chicks. This is in agreement with results shown in table 1 and figure 1. In contrast to the earlier results which showed that dried rumen and reticulum contents from sheep fed the deficient ration contained approxi-

mately 7 μg of thiamin per gram, the results shown in table 2 indicate that the dried rumen contents of cow no. 557 contained no more thiamin than basal diet 152, or less than 0.4 μg. per gram. Later assays in which dried rumen material from cow no. 557 was fed at levels of 20% and 30% substantiated this conclusion.

Despite the fact that no thiamin was detected in the rumen contents of cow no. 557 it may be seen from table 2 that her milk contained an appreciable amount of the vitamin. From the polyneuritic mortality index values for groups VI and

TABLE 2

Thiamin assay of ruminant ration, rumen contents and dried skim milk

GROUP NO.	SUPPLEMENT ADDED TO BASAL DIET 152	NUMBER OF CHICKS AT BEGINNING OF ASSAY	POLYNEURITIC MORTALITY INDEX
I	0	10	17.7
II	14% ruminant ration	10	18.5
III	7% rumen contents from no. 557 dried at pH 4.5 to 5.0	10	18.2
IV	14% rumen contents from no. 557 dried at pH 4.5 to 5.0	10	16.8
V	15% dried skim milk from no. 557	10	15.5
VI	23% dried skim milk from no. 557	10	7.0
VII	20% University Farm skim milk powder	10	6.4
VIII	50 μg. thiamin per 100 gm.	10	7.4
IX	80 μg. thiamin per 100 gm.	10	4.4

VIII the thiamin potency of dried skim milk from the experimental cow was estimated to be between 2 and 2.5 μg. per gram. From the index value of 6.4 for group VII it was estimated that the thiamin content of this sample of commercial skim milk powder was about 3 μg. per gram.

The fact that no thiamin was detected in the rumen contents while an appreciable quantity of the vitamin was secreted in the milk appeared explainable in either of two ways. First, thiamin synthesized in the rumen may have been destroyed during processing of the rumen contents or second, thiamin synthesis may have occurred in sections of the digestive tract posterior to the rumen. As described in a previous com-

munication (McElroy and Goss, '40 a) the method used in preparing cow rumen samples differed from that employed for sheep rumen samples in that the cow rumen contents were dried in an oven before a current of air at 40° to 50°C. after the addition of ethyl alcohol to make the concentration of alcohol 30% and the addition of HCl to make the pH 4.5 to 5.0, while the sheep material was dried at room temperature (not exceeding 30°C.) after the addition of enough alcohol to make the concentration about 50%. No acid was added to the sheep rumen and reticulum contents. Sheep rumen and reticulum contents treated in this way yielded a product which assayed 7 μg. of thiamin per gram, but samples of the rumen contents from cow no. 557 treated by the method used for the material from the sheep showed no more thiamin activity than

TABLE 3

Thiamin assay of dried cow rumen contents from cow no. 706

GROUP NO.	SUPPLEMENT ADDED TO BASAL DIET 152	NUMBER OF CHICKS AT BEGINNING OF ASSAY	POLYNEURITIC MORTALITY INDEX
I	0	12	18.1
II	15% rumen contents dried at pH 4.5	12	13.8
III	50 μg. thiamin per 100 gm.	12	11.2

did samples prepared in the regular way. It would therefore appear that destruction of thiamin during drying cannot be offered as a major factor in explaining the low level of the vitamin in the rumen contents of fistulated cow no. 557.

As has been previously stated, the sample of rumen contents obtained from non-fistulated cow no. 706 was not representative of the organic matter present in the rumen but as shown in table 3 the addition of 15% of this material to diet 152 served to delay the onset of fatal polyneuritis in chicks. From table 3 and the response curve of Jukes and Heitman ('40) it was estimated that this sample of rumen contents from cow no. 706 contained approximately 2.5 μg. of thiamin per gram. This figure is undoubtedly considerably lower than that which would have been found had it been possible to assay a

sample from this cow of rumen material which was free of foreign inorganic matter. Although more comprehensive study would be required to prove the point, the response of fistulated cow no. 557 to thiamin injections and the results of the thiamin assays of rumen contents from the fistulated and non-fistulated animals suggest that the presence of an artificial opening into the rumen may alter the habitat in such a way as to make it unfavorable for the growth of rumen organisms capable of synthesizing thiamin.

DISCUSSION

Since the dried rumen and reticulum contents of sheep fed a thiamin deficient ration were found to be a good source of thiamin it is concluded that under the conditions of this investigation microorganisms present in the rumen of the sheep were able to synthesize the vitamin. In view of these results it seems reasonable to assume that the development of a state of thiamin deficiency under conditions of practical sheep husbandry is very improbable, even though it is not established that thiamin synthesis will take place in the rumen of sheep regardless of the composition of the deficient diet fed.

Scheunert and Schieblich ('23) demonstrated that B. vulgatus, an obligate intestinal organism of herbivors, is capable of synthesizing vitamin B_1. Theiler, Green and Viljoen ('15) and Bechdel and co-workers ('26, '27, '28) have conducted pioneer investigations in feeding vitamin B complex deficient rations to cattle. In the main the results of our cow experiments are in agreement with those of these early investigators in indicating that thiamin may be synthesized in the rumen of the bovine and is therefore not a dietary essential for this species.

In their work with a rumen fistula cow, Bechdel, Honeywell, Dutcher and Knutsen ('28) found that after the preparation of the fistula "the heifer continued to thrive (on the ration deficient in vitamin B) and gave every evidence of functioning normally as she had done on the experimental ration

prior to the operation." While this offers no support for our suggestion that the presence of an artificial opening into the rumen may result in a decreased thiamin synthesis it does not eliminate the possibility, particularly since their ultimate vitamin assay of the rumen contents was conducted, not with the dried rumen contents, but with a concentrated alcoholic extract of rumen material which had been incubated for 5 days "in order that the bacterial flora might multiply and produce, if possible, the maximum quantity of vitamin B." One gram of their final extract represented 25.4 gm. of the original fresh fermented rumen material and the extract was fed at a level of 50% of the diet, so that the concentration of the vitamin in the original rumen contents need not necessarily have been high in order to produce the growth response which they obtained with rats.

SUMMARY

1. Four sheep were fed a ration containing less than 0.4 μg. of thiamin per gram. The dried rumen and reticulum contents of the sheep were found to contain approximately 7 μg. of thiamin per gram.

2. No thiamin was detected in the rumen contents of two fistulated cows fed the same deficient ration, but the milk of one of these cows contained between 2 and 2.5 μg. per gram on a dry basis.

3. Thiamin was detected in the rumen contents of a non-fistulated cow fed the deficient ration.

4. These results confirm and extend the work of earlier investigators in indicating that thiamin is not a dietary essential for ruminants.

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RIBOFLAVIN CONTENTS OF SOME TYPICAL FRUITS[1]

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The quantitative distribution of riboflavin in natural food materials is of interest from two points of view. The fact that riboflavin functions importantly in the tissue respiratory and perhaps other processes of both plant and animal nutrition gives scientific significance to a knowledge, as broad and as precise as is practicable, of the relative concentrations of this substance in different plant and animal tissues. The relative richness of different foods in riboflavin is also brought closer to the scientific interests and professional responsibilities of workers in nutrition by the present-day knowledge that shortage of riboflavin in the dietary is a not infrequent human nutritional deficiency disease (Sebrell and Butler, '39; Oden, Oden and Sebrell, '39; Sydenstricker, Geeslin, Templeton and Weaver, '39; Kruse, Sydenstricker, Sebrell and Cleckley, '40; Sydenstricker, Sebrell, Cleckley and Kruse, '40).

Two citrus and two pome fruits and one species of each of two other families, including the tomato as being botanically a fruit though often called a vegetable, are reported upon in this paper.

MATERIALS AND METHODS

In the case of apple and of pear, the "edible portion" was represented by a section of the fresh flesh of the fruit with its proportionate amount of skin, but without seeds. Banana was fed as sections of the fresh fruit with skin removed. Orange

[1] The aid of a grant from the Florida Citrus Commission is gratefully acknowledged.

was fed as fresh fruit with skin and seeds but not septum removed; grapefruit, after removal of skin, seeds, and septum. Tomato was fed as the mixed juice and pulp of canned tomato after passing through a coarse sieve to remove skins and seeds only. Canned grapefruit juice was also tested.

The determinations of riboflavin here reported were all made by the feeding method, using young rats essentially as described by Bourquin and Sherman ('31) with the added precaution of harnessing for complete prevention of coprophagy

TABLE 1

Riboflavin contents of certain fruits

FRUIT	NUMBER OF TEST ANIMALS	MICROGRAMS OF RIBOFLAVIN FOUND: per 100 gm. Mean ± P.E.[1]	MICROGRAMS OF RIBOFLAVIN FOUND: per 100 Calories Mean ± P.E.[1]
Apple	14	4.3 ± 1.2	7.7 ± 2.1
Banana	12	47.7 ± 4.6	48.7 ± 4.7
Grapefruit	26	20.0 ± 2.1	45.5 ± 4.8
Grapefruit juice (canned)	12	11.8 ± 1.4	(see text)
Orange	17	27.8 ± 2.0	55.6 ± 4.0
Pear	14	19.8 ± 2.8	28.3 ± 4.0
Tomato	13	37.3 ± 3.3	162.4 ± 14.3

[1] P.E. = the probable error of the mean.

as described by Page ('32), and with all tests controlled by strictly parallel (side-by-side) feedings with pure riboflavin. The 4-week test period was used.

FINDINGS AND CONCLUSIONS

The findings are summarized in table 1, as micrograms of riboflavin both per 100 gm. and per 100 Calories of the fresh fruit (the latter, however, omitted in the case of canned grapefruit juice because of differing customs as to presence or absence of added sugar).

Compared either per 100 gm. or per 100 Calories of fresh edible material, the citrus fruits (orange and grapefruit), banana, and tomato appear richer in riboflavin than the pome fruits as here represented by apple and pear. Considered with reference to the amounts presumably involved in average

human nutrition (Stiebeling and Phipard, '39) each 100-Calorie portion of citrus fruit (grapefruit or orange) or of banana contributes its full quota of riboflavin to the dietary, while the 100-Calorie portion of tomato contributes more, and that of pome fruit (apple or pear) contributes less.

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EFFECTS OF PARATHYROID DEFICIENCY AND CALCIUM AND PHOSPHORUS OF THE DIET ON PREGNANT RATS[1]

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This paper is devoted to a comparison of the course of pregnancy in normal and parathyroid-deficient rats and includes data on the composition of the blood at term. These observations are related to the endocrine deficiency and to the calcium and phosphorus content of the diet.

The choice of the albino rat (inbred Wistar strain) for these experiments has, aside from the advantages generally recognized by students of nutrition, the added and distinct advantage that in this animal complete extirpation of the parathyroids may be accomplished with a high degree of probability. The rat possesses, as a rule, two parathyroids, situated at the upper poles of the thyroid gland on the anterolateral surface of each lobe. They are well encapsulated and are removable by micro-dissection without serious damage to the thyroid or other contiguous structures. Though certain of the earlier investigators assumed the existence of accessory parathyroid tissue, there now seems to be general agreement with the conclusion of Hoskins and Chandler ('25) that the occurrence of accessories is so infrequent as to be negligible in properly controlled experiments. Aberrant parathyroid tissue, when present, is usually located cephalad to the thyroid and dorsal to the cartilage of the larynx.

[1] This work was aided by generous grants from the Mead Johnson Company, Evansville, Indiana, and approved by the Council for Pediatric Research of the American Academy of Pediatrics.

A possible limitation to the use of the rat in studies involving maternal and fetal mineral metabolism is the cartilaginous nature of the rat skeleton at birth, as compared to that of the human infant at birth. The per cent of ash of the rat pup is only about half that of the newborn infant; the calcium content per unit of body weight is only about a third, while the phosphorus content is in the neighborhood of 60%. As Cox and Imboden ('36 b) have shown, it requires approximately 21 days of nursing to raise the total ash, calcium and phosphorus of the rat pup to levels comparable to those of the human infant at birth.

These comparisons do not signify, however, that gestation per se involves less strain on the mineral stores of mother rats than obtains in human mothers, for it is only necessary to take into account that an average litter, weighing 50 gm., may be equivalent to one-fifth or even one-fourth of the maternal weight. Even though the per cent of calcium in a fetus is relatively low, the total calcium of a litter usually amounts to 5%, or more, of the total calcium of the maternal organism. In comparison, the average human infant, at birth, contains 24 to 30 gm. of calcium, which is in the neighborhood of 1.5 to 2.5% of the calcium estimated to exist in the average woman of child-bearing age.

The results of parathyroid deficiency are so significantly affected by the mineral composition of the diet that it seemed worth while to conduct our studies at different levels of calcium and phosphorus intake. The basal diet and the various experimental diets consisted of purified foodstuffs and were identical in composition with those employed by Cox and Imboden ('36 a) in their investigation of the role of calcium and phosphorus as determinants of reproductive success in normal rats. The composition of the basal diet is given in table 1.

Following the procedure given by Cox and Imboden, predetermined quantities of dicalcium phosphate and either monobasic ammonium phosphate or calcium acetate were added to the basal diet, thereby bringing the calcium and phosphorus

to the desired levels. Eight diets representing a comparatively wide range of variation of the calcium and phosphorus intake (see table 2) were employed in our studies.

Cox and Imboden ('36 a) determined reproductive success on the basis of the following criteria: (1) average weight of

TABLE 1

Composition of basal diet

	gm.
Casein (Labco, acid washed vitamin free)[1]	20.0
Lard (Swift's Silverleaf)	9.0
Yeast concentrate[2]	4.0
Wheat germ oil	1.6
Carotene solution in oil 3: 1000	0.3
Salts — Ca and P free[3]	3.1
Rice cellulose[4]	5.9
Dextrinized starch, after adding calcium-phosphorus mixtures, to make	100.0

[1] Purchased from Casein Company of America.

[2] We are indebted to the Mead Johnson Company, Evansville, Indiana, for the large quantities of yeast concentrate used in this and subsequent studies.

[3] Composition: NaCl 24.50, $MgSO_4$ 9.14, $KHCO_3$ 59.20, KCl 3.84, Fe citrate 2.94, $CuSo_4$ 0.32, $MnSO_4$ 0.04, KI 0.02%.

[4] We are indebted to Mr. E. R. Harding of the H. J. Heinz Company, Pittsburgh, Pennsylvania, and to Mr. C. M. Williams of the Lake Charles Products Corporation, Lake Charles, Louisiana, for supplying, without charge, all the rice cellulose used in this and subsequent studies.

TABLE 2

Levels and ratios of calcium and phosphorus in experimental diets

DIET NO.	PER CENT Ca	PER CENT P	Ca/P RATIO
7	0.49	0.49	1.0
8	0.49	0.735	0.66
10	0.49	2.450	0.2
12	0.735	0.490	1.5
16	1.225	0.245	5.0
19	1.225	1.225	1.0
26	0.017	0.245	0.07
27	0.122	0.245	0.5

the young at 21 days; (2) their percentage ash; and (3) the change in weight of the mother during the reproductive cycle. Owing to the fact that our experiments required the sacrifice of a considerable proportion of the mother rats and their offspring at the time of delivery, we could not employ these

criteria. Moreover, in parathyroid-deficient mothers, milk production was depressed to a point which made it impossible to carry a litter through a 21-day period of lactation successfully. Accordingly, the comparisons have been based on the following considerations: (1) per cent of fertile matings; (2) average number per litter; (3) average weight of rat pups at birth. Data to be submitted in subsequent papers in this series will contribute additional criteria for comparing reproductive success between normal and parathyroid-deficient rats maintained at different levels of intake of calcium and phosphorus.

Per cent of fertile matings. Conforming to the practice at The Wistar Institute (Greenman and Duhring, '31), the rats were mated only after they had attained an age of 110 to 120 days. The routine procedure for breeding in our experiments consisted in accustoming the rat to a particular diet, then exposing the female to its mate for a period of 7 days. Since estrus recurs about every 5 days this assured successful copulation in the majority of instances.

Table 3, column 4, contains comparative data of the per cent of fertile matings in normal and parathyroid-deficient rats kept on various diets. Attention is directed particularly to diets 7, 26, and 27, as these embraced larger numbers of experiments. On diet 7, which Cox and Imboden found to be conducive to maximum reproductive success in normal rats, thirty-five animals were mated a total of 107 times; 65 of the matings proved fertile. The per cent of fertile matings (60.1) was lower than that (76.8) recorded by Cox and Imboden for this diet. The difference may be explained partly on the basis that Cox and Imboden used rats which had previously gone through one successful mating on stock diet. Out of the thirty-five rats on diet 7 in our series, three proved sterile after repeated matings. If correction were made by excluding these three animals, the percentage of fertile matings in this group would be raised to 67%. On diet 26 the percentage fertile matings was 46.7, compared to 50.0% obtained by Cox and Imboden. In thirteen normal rats on diet 27, the per cent fertile matings was 81.5, compared to 68.4% found by Cox and

TABLE 3

Reproductive success in normal and parathyroid-deficient (P-D) rats on various diets

DIET NO.	NORMAL CONTROLS, OR PARATHYROID-DEFICIENT (P-D)	NUMBER IN GROUP	FERTILE MATINGS	NUMBER PER LITTER				WEIGHTS OF LITTERS				AVERAGE WEIGHTS OF RAT PUPS				INDICES OF REPRODUCTIVE SUCCESS IN P-D RATS IN RELATION TO CORRESPONDING CONTROLS
				Min.	Max.	Averages	S.D.[4]	Min.	Max.	Averages	S.D.[4]	Min.	Max.	Averages	S.D.[4]	
			%					*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
7	Normal	35	65/107 = 60.1 (76.8)[1]	3	16	9.3 (30)[2]	3.1	16.9	76.2	49.62	15.38	4.03	7.10	5.45	0.67	
	P-D	58	55/144 = 38.2	4	13	8.6 (40)	2.3	14.7	54.01	29.19	8.20	1.98	5.38	3.49	0.83	0.733
8	Normal	4	10/10 = 100 (79.2)	10	15	11.5 (6)	1.9	40.00	66.6	56.67	9.43	4.00	5.74	4.95	0.67	
	P-D	4	6/19 = 31.6	7	8	7.5 (2)	0.7	26.5	41.2	33.87	10.38	3.31	5.88	4.58	1.89	0.664
10	Normal	6	14/20 = 70 (71.4)	6	13	10.0 (7)	2.6	27.96	62.53	48.85	12.97	4.66	5.28	4.88	0.23	
	P-D	6	1/24 = 4.2			9.0 (1)	—	—	—	20.34	—	—	—	2.26	—	0.475
12	Normal	6	12/13 = 92.3 (84.6)	7	13	9.9 (7)	2.3	43.05	72.22	55.47	11.65	4.94	6.43	5.69	0.60	
	P-D	10	15/31 = 48.4	6	11	8.8 (6)	1.7	28.44	53.60	40.01	19.82	3.50	5.36	4.53	0.59	0.736
16	Normal	9	17/26 = 65.4 (70.6)	4	12	8.2 (13)	2.0	23.56	63.72	42.62	9.67	4.4	6.0	5.24	0.49	
	P-D	18	30/55 = 54.5	5	12	8.4 (15)	2.8	26.91	59.40	41.60	9.14	3.0	5.75	4.62	0.72	0.913
19	Normal	5	7/7 = 100 (82.4)	5	11	10.7 (7)	1.6	19.4	55.66	55.47	7.65	4.78	5.87	5.19	0.36	
	P-D	7	5/6 = 83	8	13	9.2 (5)	2.5	41.2	64.5	31.86	14.66	1.86	5.06	3.50	1.15	0.788
26	Normal	16	28/60 = 46.7 (50.0)	5	14	9.5 (14)	1.5	13.65	75.6	45.12	16.96	2.73	6.15	5.14	0.98	
	Mated on 7[3]	6	10/11 = 90.9	8	12	10.0 (10)	1.4	40.00	68.88	52.40	8.02	4.9	5.74	5.24	0.30	
	P-D	23	3/90 = 3.3			4.0 (1)				20.61	—	—	—	5.15	—	0.495
	Mated on 7[3]	12	12/49 = 24.5	2	14	7.6 (10)	3.9	8.84	35.56	23.24	9.85	2.14	4.68	3.36	0.96	0.557
27	Normal	13	22/27 = 81.5 (68.4)	8	15	11.5 (13)	2.3	40.00	75.48	56.15	11.21	3.05	6.29	4.95	0.87	
	P-D	17	27/92 = 29.35	2	12	6.4 (12)	3.1	10.94	31.00	21.01	6.45	2.13	5.88	3.67	1.11	0.552

[1] Figures enclosed by parentheses in this column denote the values for per cent fertile matings obtained with similar diets by Cox and Imboden ('36a).

[2] Figures in parentheses in this column denote the number of litters on which the averages were based.

[3] Because of inability of parathyroid-deficient rats to breed on diet no. 26, twelve such animals and six controls were mated on diet no. 7, then transferred to diet no. 26, on which they were kept until the conclusion of the gestation period.

[4] S.D. = standard deviation.

Imboden. On diets nos. 10, 12, and 16 there was even closer agreement between our results and those of Cox and Imboden. On diets 8 (four rats, ten matings) and 19 (five rats, seven matings), the percentage fertility in the normal, unoperated rats was, in both instances, 100%.

That parathyroid deficiency is accompanied by marked reduction in fertility is evident from the data in table 3. This was much more conspicuous on certain diets than on others. Thus, in the parathyroid-deficient group on low-calcium diet no. 26, out of ninety matings in twenty-five animals, there were only three known pregnancies (3.3%). However, when parathyroid-deficient rats which after repeated matings had proved sterile on diet no. 26 were changed to some other diet, such as no. 16 or no. 7, a significant number became pregnant. To illustrate, one such group of twelve rats, repeatedly though unsuccessfully mated on diet no. 26, were bred on diet no. 7, then restored to diet no. 26, on which they were kept for the greater part (14 to 19 days) of the gestation period. In this way the percentage of fertile matings was increased to 24.5. The highest incidence of fertile matings in the parathyroid-deficient group, 54.5%, occurred on diet no. 16 (Ca, 1.225%; P, 0.245%; Ca/P ratio, 5.0). The lowest, 3.3%, occurred on diet no. 26 (Ca, 0.017%; P, 0.245%; Ca/P ratio, 0.07). The second lowest, 4.2%, occurred on diet no. 10 (Ca, 0.49%; P, 2.45%; Ca/P ratio, 0.2).

Average number per litter. Greenman and Duhring ('31), describing the experience at The Wistar Institute, state that the number of young per litter varies greatly, but that under favorable conditions the average may reach seven or eight, or even more. The average for first litters is given as 9.97, that for fifth litters as 6.0. Reference to table 3 shows that for the unoperated rats the average number per litter varied from 8.2 on diet no. 16 to 11.5 on diets 8 and 27. For the parathyroidectomized rats the averages were distinctly lower than those for the normals on all diets excepting 16. Although small litters occurred somewhat more frequently among the parathyroid-deficient rats than among the normals, the range of

variation in the numbers per litter was not strikingly different in the two groups of animals.

Average weight of rat pups at birth. Several factors are known to influence the birth weight of rats. Increased maternal age and weight are associated with heavier birth weights. Newborn males weigh on the average 0.3 gm. more than females. Increase in the number per litter tends to diminish the birth weight. The nutritional status of the mother is also important, an inadequate or unbalanced diet being associated with low birth weight. Season may be a factor, as shown by statistics obtained at The Wistar Institute (Greenman and Duhring, '31).

The data for normal birth weights recorded in the literature show considerable variation. Thus, the majority of the values cited by Donaldson ('24) are below 5 gm., while those given by Greenman and Duhring ('31) range from 5.33 to 5.84 gm. for females and from 5.6 to 5.95 gm. for males. Jackson ('40) recently reported his experience with two diets. One group of ten female rats, kept on a commercial dog food,[2] produced forty-two litters, averaging 9.93 rats per litter, with an average birth weight of 5.20 gm. A second group of ten rats, kept on McCollum diet 1, produced thirty-eight litters, averaging 8.21 rats per litter, with an average birth weight of 5.23 gm. Cox and Imboden ('36 a) give 5.37 gm. as the average birth weight of 239 first-litter rats, with the mothers kept on a stock diet, and 5.12 gm. for second-litter rats on various experimental diets. In our experiments the birth weights of the young from normal mothers kept on diet no. 7 (279 pups) averaged 5.45 gm. The highest birth weights, average of 7.10 gm., occurred in a litter of three (a fifth litter); the lowest average value, 4.03 gm., occurred in a first litter of twelve. The largest litter on diet 7, a first litter, consisted of sixteen young with an average weight of 4.76 gm.

Diet seemed to be a much more significant factor in the parathyroid-deficient group than in the normals. In the normal controls the lowest average birth weight, 4.88 gm., was ob-

[2] Purina dog chow.

tained on diet no. 10 (seventy young); the highest average value for the whole group on any diet, 5.69 gm., occurred on diet no. 12 (seventy young). In the parathyroid-deficient group the lowest average value, 2.26 gm., was obtained on diet no. 10 (one litter of nine), while the highest value, 4.62 gm., occurred on diet no. 16 (128 young). On diet no. 12, the average birth weight in the parathyroid-deficient group was 4.53. From the data in table 3 it is apparent that parathyroid deficiency in the mothers produced retardation of fetal growth and that this effect was more marked on some diets than on others.

INDICES OF REPRODUCTIVE SUCCESS

The ratio: $\frac{\text{per cent fertile matings in parathyroidectomized rats}}{\text{per cent fertile matings in normal controls}}$ varied from 0.139 on diet no. 26 to 0.834 on diet no. 16. The corresponding ratios for the average number per litter varied from 0.556 on diet no. 27 to 1.023 on diet no. 16, while the ratios for the average birth weight varied from 0.463 on diet no. 10 to 0.926 on diet no. 8. The sum of the ratios for any particular group divided by 3 provides an index which may be used for rough comparisons of reproductive success on the various diets employed in the study (see table 3). The lowest index was obtained on (high phosphorus) diet no. 10, 0.475, and the highest on (high calcium-low phosphorus) diet no. 16, 0.913. It is of interest that the latter diet, though rachitogenic and therefore unsatisfactory under ordinary circumstances, was singularly effective in promoting reproductive success in the parathyroid-deficient rat.

However, neither the individual factors considered so far nor the indices just mentioned describe adequately the effects of hypoparathyroidism on reproduction. Long ago, Halsted (1896) noted that partly "thyroidectomized" dogs which had become pregnant developed tremors and convulsions at term. Carlson ('13) found that thyro-parathyroidectomy in dogs in the latter stages of pregnancy resulted in a high mortality. Thus, eight out of fifteen animals died in 12 to 24 hours after the operation. The effects of parathyroid deficiency in pregnant animals have been recorded also by Luckhardt and

Rosenbloom ('22), by Dragstedt, Sudan and Phillips ('24), Kozelka, Hart and Bohstedt ('33) and by others. Bodansky and Cooke ('37) reported the results of thyro-parathyroidectomy in rats. Out of sixty-two animals, twenty-four succumbed at the conclusion of the first pregnancy, although they had appeared in normal health until 2 or 3 days before term. Eighteen others did not survive subsequent pregnancies. That the symptoms developing at term were due largely to parathyroid deficiency has been established by extending the studies to rats deprived of the parathyroids only.

The course of pregnancy in the parathyroidectomized rat has been summarized previously by Bodansky and Duff ('39). When kept on satisfactory rations these animals may become pregnant and thrive until toward the end of gestation. However, from 2 to 4 days before term slight tremors of the paws develop, these being more apparent when the rat is held up. The tremors become progressively more pronounced, the body is stiffened and the tail becomes rigid. The rat moves stiffly and with apparent difficulty even on a flat surface. Anorexia is a prominent symptom. Despite these severe manifestations the rat may improve, go through delivery and recover. Or, the downward course may proceed in one of two ways. Rarely, the tremors become more and more severe, ultimately assuming tetanic convulsive proportions; the rat loses consciousness and death occurs frequently within about 12 to 18 hours after onset of symptoms. Much more commonly the tremors cease; the rat becomes more and more quiescent and then lethargic. It can be roused only by pronounced stimuli. Meanwhile the body temperature drops, and finally the rat dies in coma.

These events may commence after the onset of labor (appearance of blood at the vagina). As a rule death occurs before any of the young have been delivered, but occasionally it may follow the delivery of part of the litter. A few of the animals in our series died after completing delivery, but in these, labor was markedly prolonged (from 1 to 2 days). In parathyroid-deficient rats the onset of labor is frequently de-

layed beyond the normal gestation period. As a rule the duration of labor is also prolonged.

Such manifestations have not been observed among the unoperated control rats, or among the stock animals in our colony.

BLOOD CHEMISTRY

Serum calcium. The relation of the symptoms to the low serum calcium at term has been investigated (table 4). In the control rats fed diet no. 7 the serum calcium during parturition averaged 9.0 ± 1.09 mg., whereas in the parathyroidectomized animals on the same diet, the average was 4.65 ± 1.06. Similar relations held for other diets, excepting (high calcium) diet no. 16. On this diet, which was clearly rachitogenic (Ca/P ratio = 5), the serum calcium of the parathyroid-deficient rats during labor varied from 7.85 to 14.3 mg., the average being 11.50 ± 1.88. This was only slightly below the average of 11.77 ± 1.51 recorded for the control animals on the same diet. It may be noted further that this was the only diet in our series which did not affect adversely the course of pregnancy and parturition in parathyroidectomized rats. That this was related to the relatively high calcium intake and the maintenance of a normal level of calcium in the blood was demonstrated as follows. Rats which had successfully completed one or more pregnancies on diet no. 16, and which were shown to have normal serum calcium during labor, were transferred to diet no. 7. Invariably the succeeding pregnancy ended abnormally; the rat developed the characteristic symptoms of parathyroid deficiency and in those instances in which blood was obtained for analysis, the serum calcium was found invariably to be low (5.2 mg. per 100 cc., or less).

The lowest serum calcium, 1.94 mg. per 100 cc., occurred during labor in a parathyroidectomized rat on diet no. 27.

Inorganic phosphorus. The concentrations of phosphorus in the blood serum showed wide variation but were, in general, higher in the parathyroid deficient rats than in the controls. Thus, in the unoperated rats fed diet no. 7 the values varied

TABLE 4

Serum calcium, phosphorus and phosphatase of normal and parathyroid-deficient pregnant rats at term

NORMAL OR PARATHYROID-DEFICIENT (P-D)	DIET NO.	CALCIUM IN MG. PER 100 CC.					INORGANIC P IN MG. PER 100 CC.					PHOSPHATASE UNITS				
		Analyses	Min	Max.	Averages	S.D.[2]	Analyses	Min.	Max.	Averages	S.D.[2]	Analyses	Min.	Max.	Averages	S.D.[2]
Normal	7	27	7.76	11.00	9.10	1.09	25	2.6	8.0	4.67	1.64	23	3.54	36.7	17.4	9.93
P-D		39	2.37	7.00	4.65	1.06	31	2.8	27.8	7.81	4.77	26	3.64	30.6	9.19	5.22
Normal	8	5	7.5	10.6	8.80	1.13	5	3.95	11.2	6.07	2.94	5	11.64	26.8	21.64	5.93
P-D		2	3.1	3.7	3.40	0.42	1	—	—	8.0	—	1	—	—	6.8	—
Normal	10	6	7.2	12.2	9.88	1.76	5	4.38	6.0	4.97	0.74	5	12.3	35.7	23.7	9.52
P-D		1	—	—	3.20	—	1	—	—	5.25	—	1	—	—	15.2	—
Normal	12	8	7.6	11.14	9.36	1.06	7	2.45	10.37	5.66	2.86	7	8.07	38.1	24.78	9.70
P-D		6	3.4	5.3	4.5	0.73	4	2.50	10.10	7.39	3.53	4	2.67	23.5	11.94	9.12
Normal	16	8	10.0	15.0	11.77	1.51	6	0.55	5.74	2.52	1.74	6	7.16	35.25	13.30	10.84
P-D		13	7.85	14.3	11.50	1.88	8	1.28	2.49	1.90	0.55	8	4.22	30.10	13.08	9.34
Normal	19	5	5.35	9.16	8.09	3.16	5	4.26	6.18	5.26	0.74	5	8.03	14.08	10.37	2.36
P-D		4	3.27	4.98	3.85	0.77	4	4.97	12.67	8.34	3.19	2	5.21	6.03	5.62	0.58
Normal	26	17	4.7	10.80	8.18	1.86	16	1.85	7.50	3.90	1.08	13	2.48	22.08	12.58	5.95
P-D[1]		10	2.85	5.33	4.06	0.79	9	3.03	7.23	5.44	1.31	9	4.22	27.50	10.31	7.36
Normal	27	12	7.07	11.55	9.14	1.49	10	2.65	6.56	5.13	1.16	9	5.76	27.16	20.45	9.22
P-D		11	1.94	6.00	4.24	1.18	11	5.92	19.7	10.76	4.59	9	2.72	30.25	14.39	8.71

[1] Because of inability of parathyroid-deficient rats to breed on diet no. 26, nine of these animals were mated on diet no. 7, then transferred to diet no. 26 on which they remained for a period varying from 14 to 19 days, until the conclusion of gestation.

[2] S.D. = standard deviation.

from 2.6 to 8.0 mg. (average 4.67 mg.) per 100 cc., while in the parathyroidectomized rats on the same diet the values varied from 2.8 to 27.8 mg. (average 7.81 mg.) per 100 cc. These observations are in general agreement with the accepted view (Greenwald and Gross, '25; Logan, '39, and others) that the parathyroid hormone regulates the excretion of inorganic phosphate and therefore affects its concentration in the blood. The lowest values for serum inorganic phosphorus were observed on diet no. 16 (Ca, 1.225%; P, 0.245%). The high Ca/P ratio was evidently a factor in maintaining the serum inorganic phosphorus at a low level not only in the normal controls, but in the parathyroid-deficient rats as well.

Serum phosphatase. The phosphatase activity of the serum, and more particularly the phosphatase of osseous origin, is believed to be an expression of the specific reactivity of the bone (Bodansky and Jaffe, '34). From the data in table 4 we note that the phosphatase varied over a wide range in each group, but that the average values were, with the exception of the rats on diet no. 16, higher in the normal controls than in the operated rats. Owing to the variability of the data in different individuals of the same group, the probable significance of single results cannot be defined with certainty. However, the data indicate that in general the capacity for cellular activity in bone tended to be less in the parathyroid-deficient rats than in the normal animals, a conclusion which is in line with existing knowledge of the relation of the parathyroids to bone metabolism.

It is interesting to note that diet no. 16, owing to its high calcium-phosphorus ratio, is definitely rachitogenic and thus promotes demineralization of the maternal skeleton and prevents normal fetal calcification. At the same time, this diet is effective in abolishing the discrepancies between normal and parathyroidectomized pregnant rats with respect to serum calcium, phosphorus and phosphatase.

SUMMARY

Parathyroid deficiency in the rat is associated with markedly diminished fertility, moderate reduction in the number per litter and a significant reduction in the average birth weight. These effects are more conspicuous on diets either low in calcium or high in phosphorus.

Parathyroid deficiency in the pregnant rat is also characterized by prolongation of the gestation period, tetany at term, prolonged labor and high rates of maternal and fetal mortality. These abnormal manifestations appear to be related to the marked lowering in serum calcium.

The concentration of inorganic phosphate in the blood serum of operated animals is usually above that of the controls on the same diet. The phosphatase is usually lower than in the controls, indicating that the capacity for cellular activity is less in the bones of the parathyroid-deficient rat than in those of the normal animal.

In parathyroid-deficient rats fed a diet abnormally high in calcium and relatively low in phosphorus (diet no. 16 of Cox and Imboden), the values for serum calcium, phosphorus and phosphatase fell within the same limits of variation and showed approximately the same averages as in the normal controls. The abnormal manifestations of late pregnancy were likewise abolished and the differences in per cent fertility, average number per litter and average birth weight were greatly minimized.

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THE DISTRIBUTION OF PANTOTHENIC ACID IN CERTAIN PRODUCTS OF NATURAL ORIGIN

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The distribution of pantothenic acid in a number of foods was reported previously in several communications (Jukes and Lepkovsky, '36; Jukes, '37; Jukes and Richardson, '38) in terms of "filtrate factor units" as determined by assay with chicks. Subsequently it was observed that one such "unit" corresponded to 14 micrograms of pantothenic acid (Jukes, '39). The present report summarizes the results of biological assays of various materials by the chick method previously described (Jukes, '37).

The use of chicks for the assay is cumbersome by comparison with microbiological methods (Pennington, Snell and Williams, '40; Strong, Feeney and Earle, '40). However, from the standpoint of nutrition, the chick assay method is perhaps advantageous in that it parallels the conditions encountered during alimentation. These observations do not alter the probability that the microbiological assay method will almost entirely replace the chick assay method in the future.

EXPERIMENTAL

The basal diet (80G) was previously described (Jukes, '39). In recent assays, the "positive control diet" consisted of 100 gm. of diet 80G plus 6 mg. of synthetic (dl) pantothenic acid. It has been shown (Babcock and Jukes, '40) that synthetic (dl) pantothenic acid has one-half the activity for chicks of that possessed by natural pantothenic acid. The entire biological activity of synthetic pantothenic acid has been

shown by Stiller and co-workers ('40) to be due to the dextro-rotatory isomer. One chick "filtrate factor" unit therefore corresponds to 14 μg. of dextro-rotatory pantothenic acid and the levo-rotatory form is without biological activity. The level of synthetic pantothenic acid fed in the positive control diet was slightly more than twice the amount necessary for maximum growth. Assay diets were supplemented when possible with levels of the test material sufficient to produce between 60 and 80% of the maximum growth response.

The care of chicks has been described previously (Jukes, '37). Following hatching, the chicks were kept on a "normal" diet for 1 week. The "normal" diet used was Chick Mash 1 (Almquist, Jukes and Newlon, '38). Following this, diet 80G was fed for a depletion period of about 2 weeks. The chicks were then weighed, divided into weight classes, separated into groups, and fed the test diets for an assay period of 9 to 14 days. The assay was usually terminated by the time the positive control group had gained an average of from 60 to 90 gm.

The calculation of the pantothenic acid content of the supplements was explained previously (Jukes and Lepkovsky, '36) and is as follows:

Pantothenic acid content of supplement in micrograms per gram =

$$\frac{(\text{Gain on test diet } \textit{minus} \text{ gain on basal diet}) \times 14}{(\text{Gain on positive control diet } \textit{minus} \text{ gain on basal diet}) \times (\text{Amount of supplement in 1 gm. of test diet})}$$

This revised calculation is based on the finding that one chick "filtrate factor" unit corresponds to 14 μg. of natural pantothenic acid (Jukes, '39). Similar results for the conversion factor have been obtained with synthetic pantothenic acid. Supplements to be tested were incorporated with the heated diet as explained previously (Jukes, '39). Foods containing large amounts of water were mixed with the diet and passed through a food-chopper or rubbed through a sieve. The mixture was then dried in thin layers at room temperature and re-weighed.

The data from a typical assay are presented in table 1.

From table 1 it may be calculated that the pantothenic acid contents of dried carrots and dried broccoli are respectively 13 and 46 μg. per gram.

The results of a number of assays are presented in table 2. Also included are some previously published data which have been recalculated in terms of micrograms of pantothenic acid per gram of supplement. The materials tested are divided into arbitrary classes with respect to pantothenic acid content. For classification, the pantothenic acid content is expressed in micrograms per gram of dry material. Many of the foods

TABLE 1

Chicks hatched and placed on normal diet on June 6. Placed on heated diet on June 13. Weighed and segregated into groups of eight on June 26

GROUP	SUPPLEMENT TO 100 GM. DIET 80G	WEIGHT IN GRAMS			NUMBER OF CASES OF DERMATITIS	
		June 26	June 30	July 6	June 26	July 6
1	None	85	88	90	3	7
2	19 gm. dried carrots	83	91	102	3	5
3	17 gm. dried broccoli	86	106	135	3	0
4	6 mg. racemic pantothenic acid	85	114	168	4	0

tested were high in water. These are expressed in terms both of fresh material and dry material. Other products, such as grains and nuts, are expressed in terms of the undried materials.

DISCUSSION

Wide variation was found to exist in the pantothenic acid content of various samples of the same product. For this reason, results in table 2 which are based on small numbers of assays may be less reliable than results in which larger numbers of assays were involved. In some instances, such as eggs, the variation may be due to the diet of the animal concerned (Lepkovsky et al., '38). The cereal grains appeared to be less variable than a number of the other products. Most of the fruits tested were poor sources.

TABLE 2

Distribution of pantothenic acid in certain products of natural origin, in terms of micrograms of pantothenic acid per gram of material. All common human foods are expressed on the basis of the edible portion

MATERIAL	PANTOTHENIC ACID CONTENT IN MICROGRAMS PER GRAM				NUMBER OF ASSAYS
	Dry material		Undried basis		
	Range	Average	Range	Average	
Excellent sources (more than 28 micrograms per gram of dry material)					
Brewers' dried yeast	140 to 350	200			10
Liver, mammalian	100 to 270	180	25 to 60	40	4
Egg yolk	100 to 200	125	50 to 100	63	4
Eggs	32 to 190	108	8 to 48	27	37
Commercial "liver meal"			100 to 110	105	2
Broccoli leaves and blossoms		87		14	1
Cane molasses, "black strap"	5 to 100	70			11
Peanut meal			45 to 63	53	3
Whey	36 to 85	60	2.4 to 5.7	4	17
Broccoli		46		11	1
Buttermilk, churned	35 to 56	46	3.5 to 5.6	4.6	4
Sweet potatoes	31 to 46	38	10 to 12	11	2
Lean beef		38		10	1
Skim milk	21 to 43	36	2.1 to 4.3	3.6	9
Zucchini squash		35		3	1
Kale	23 to 36	30	2.3 to 3.6	3	2
Good sources (14 to 28 micrograms per gram of dry material)					
Canned salmon		28		7	2
Irish potatoes		28		6.5	2
Taro root		28		7	1
Alfalfa	3 to 40	25			12
Wheat bran			20 to 30	24	4
Canned pumpkin		23		4	1
Whole milk	10 to 32	22	1.3 to 4.2	2.8	4
Rice bran			15 to 27	22	3
Split peas			20 to 22	21	2
Tomatoes		20		1	2
Cowpeas			17 to 20	18	2
Soybean meal			8 to 22	14	4
Cottonseed meal				14	2
Moderate sources (10 to 14 micrograms per gram of dry material)					
Carrots		13		2	1
Jerusalem artichoke		13		4	1
Rolled oats				11	2
Wheat				11	2
Wheat middlings			10 to 11	10	2
Barley				10	2

TABLE 2 — *continued*

MATERIAL	PANTOTHENIC ACID CONTENT IN MICROGRAMS PER GRAM				NUMBER OF ASSAYS
	Dry material		Undried basis		
	Range	Average	Range	Average	
Poor sources (4 to 10 micrograms per gram)					
Spinach		10		1.2	2
Milo				9	2
Onion	8 to 11	9	1 to 1.4	1.2	2
Yellow corn			7 to 10	8	3
English walnuts				8	1
Wheat germ			3 to 12	7	4
Orange		7		0.7	2
Sesame meal				6	2
Polished rice				4	2
Banana		4		0.7	2

Other moderate to poor sources, all giving negative results in the chick assay at the level fed

SOURCE	LEVEL FED (GM. DRY FOOD PER 100 GM. DIET)	UNDRIED EQUIVALENT	PANTOTHENIC ACID CONTENT, MICROGM. PER GM. ON DRY BASIS (EXCEPT [1])
Ladino clover	8	51	Less than 1.2
Blood meal		8	" " 1.2 [1]
Canned green beans	9	80	" " 1.1
Egg white	9	80	" " 1.1
Linseed meal		10	" " 1.0 [1]
Coconut meal		10	" " 1.0 [1]
Barley shoots	10	88	" " 1.0
Lawn clippings	10	40	" " 1.0
Broccoli stems	12	89	" " 0.9
Asparagus	12	89	" " 0.9
Commercial casein		12	" " 0.9 [1]
White turnips	13	130	" " 0.8
Canned green peas	14	54	" " 0.7
Prunes		20	" " 0.6 [1]
Raisins		20	" " 0.6 [1]
Fish meal		20	" " 0.6 [1]
Meat scrap		20	" " 0.6 [1]
Canned peaches	23	156	" " 0.5
Hempseed meal		25	" " 0.4 [1]
Canned pineapple juice	26	148	" " 0.4
Beets	27	141	" " 0.4
Apples	32	195	" " 0.3
Almonds		40	" " 0.3 [1]

[1] Calculated on undried basis.

Several water-soluble vitamins, namely, thiamin, riboflavin, nicotinic acid, pyridoxine and pantothenic acid, are grouped under the term "vitamin B complex." It has long been recognized that the members of this group are commonly associated with each other in nature, and in recent years the discovery of certain of the biochemical functions of these vitamins has made their widespread occurrence in living tissues readily understandable. It is important to recognize, however, that the relative proportions of the B-complex vitamins differ widely in various foods. This observation may play a part in the "balancing" of diets. The following data are illustrative:

TABLE 3

Illustrating the variation in the distribution of certain members of the vitamin B complex as compared with the nutritional requirements of chicks

	PARTS PER MILLION		
	Thiamin	Riboflavin	Pantothenic acid
Chick requirement in diet	1.5	2.7	14
Egg (dry basis)	4	12	108
Liver (dry basis)	12	100	180
Cow's milk (dry basis)	2.5	18	22
Wheat	5	1	11
Wheat bran	6	4	24
Wheat germ	40	8	7

For example, from table 3 it may be seen that egg, liver or milk will supply the dietary requirement of the chick for thiamin, riboflavin and pantothenic acid. The three cereal products listed will supply the thiamin requirement but in some instances are deficient in riboflavin or pantothenic acid. Comparatively large amounts of riboflavin and especially pantothenic acid are present in two foods, egg and milk, which are intended for the nutrition of young animals. The data in table 3 for the thiamin content of the foods listed were obtained from recent literature on the subject. The other figures were from experimental results in this laboratory.

The question of secondary deficiencies in diet 80-G is of importance in the assay method. Observations in this labora-

tory have indicated that pantothenic acid, when added to the diet at a level of 14 or more parts per million of diet, will produce a growth response in the test as great as that produced by crude sources of pantothenic acid, such as rice bran filtrate, when these sources are added to the diet at the level sufficient for maximal growth. This does not exclude the possible existence of other nutritional essentials in rice bran and other filtrates. The crude materials, such as commercial casein and whey adsorbate, present in diet 80-G are thought to provide some of these unknown factors. The whey adsorbate should supply at least 400 μg. of riboflavin per 100 gm. of diet. The above conclusions are not necessarily valid if changes are made in the heated diet, or if prolonged assay periods are used. An assay period of from 7 to 9 days is usually satisfactory, although 14 days has been used in many of the assays reported in table 2, especially in the case of the earlier data. Recent results have indicated that the method gives reasonable agreement with the lactobacillus casei assay method when applied to crude materials.

SUMMARY

1. The pantothenic acid content of a number of foods and other products of natural origin was measured by biological assay with chicks. Data are presented for sixty-eight products, involving more than 200 bio-assays.

2. The dietary requirement of the chick for thiamin, riboflavin, and pantothenic acid is compared with the varying distribution of these vitamins in certain foods.

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THE EFFECT OF DIET ON THE PANTOTHENIC ACID CONTENT OF EGGS[1]

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While it is well established that the pantothenic acid content of the tissues of growing chicks decreases when they are restricted to a diet deficient in this constituent (Snell et al., '40) evidence on the influence of the amount in the diet on the level in the eggs is limited and inconclusive. Lepkovsky et al. ('38) reported that pantothenic acid, under the conditions studied, appears to exercise no function in maintaining normal egg production. On the other hand Bauernfeind and Norris ('39) presented evidence that pantothenic acid is necessary for normal hatchability. On the basis of hatchability data, they concluded that the pantothenic acid content of eggs can be influenced by the amount in the diet.

Since more refined methods for the determination of pantothenic acid (Pennington et al., '40) are now available it was of interest to determine if and to what extent the pantothenic acid content of the diet influences the amount in the eggs.

EXPERIMENTAL

For the data on eggs from hens receiving a deficient diet, four White Leghorn hens approximately 8 months of age were used. These birds had received since hatching a diet consisting of yellow corn 74.5, purified casein 18, salts mixture 7.5,

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riboflavin 250 μg. per 100 gm. of diet, and cod liver oil to furnish 900 U.S.P. units of vitamin A and 150 A.O.A.C. chick units of vitamin D per 100 gm. of diet. Chicks fed this diet developed the typical dermatitis from which they recovered when the diet was supplemented with a crude preparation of pantothenic acid at a rate calculated to furnish 1.4 mg. of the pure acid per 100 gm. The four hens were among those that survived the deficiency and eventually reached approximately normal size and appearance. Their egg production during the 3 months preceding these studies, while somewhat irregular, had at various times reached as many as five to six eggs per week.

The normal eggs were from hens fed a stock diet having a percentage composition of milo 40, oats 10, wheat shorts 20, fish meal 6, cottonseed meal 6, soybean oil meal 6, alfalfa leaf meal 5, dried whey 3, oyster shell 2, bone meal 1, sodium chloride 1, and cod liver oil to furnish 600 U.S.P. units of vitamin A and 80 A.O.A.C. chick units of vitamin D per 100 gm. of diet in addition to that present in the feeds. This diet contained 1.9 mg. of pantothenic acid per 100 gm. as compared with 0.44 mg. for the deficient diet.

The pantothenic acid was determined by the bacterial method of Pennington et al. ('40). Four eggs were collected from each of the hens on the deficient diet and analyses made on the combined whites and yolks from two eggs. Since the values obtained on each set of two eggs were in good agreement, only the averages of the two values are recorded in table 1. The data from the hens on the stock diet are the values obtained from the analyses of the combined whites and yolks, respectively, from two eggs.

The data in table 1 show that the amount of pantothenic acid in the eggs is dependent upon the amount in the diet. The whites of the eggs from the hens on the deficient diet contained an average of 0.41 μg. of pantothenic acid per gram and the yolks 9.66 μg. The corresponding figures for the eggs from the hens on the stock diet are 0.76 μg. for the whites and 46.6 μg. per gram of yolk. These figures for normal eggs, though

slightly lower, are of the same order as the values calculated from the data of Jukes ('37) for egg white and yolk on the basis that 1 chick filtrate factor unit corresponds to 14 μg. of pantothenic acid (Jukes, '39). Such differences are not unexpected since as our data show, the amount in the egg is dependent upon the level in the diet.

To study the effect of supplementing the deficient diet with pantothenic acid 8.4 mg. of *dl*-sodium pantothenate (Williams et al., '40) (4.2 mg. of the active isomer: Stiller et al., '40; Babcock and Jukes, '40) was added to each 100 gm. of feed.

TABLE 1

Pantothenic acid content of eggs from hens on basal stock diets

NUMBER	DIET	WHITE	YOLK	WHOLE EGG (WITHOUT SHELL)
		μg./gm.	*μg./gm.*	*μg./100 gm.*
63	Deficient	0.38	11.5	435
64	"	0.34	7.3	254
65	"	0.33	7.4	289
66	"	0.60	12.5	467
Average		0.41	9.66	361
43	Stock	0.91	45.3	1730
45	"	0.59	35.6	1270
46	"	0.73	45.5	1896
47	"	1.00	48.5	1850
48	"	0.87	68.5	2485
55	"	0.47	37.5	1170
Average		0.76	46.8	1732

This is three times the daily requirement for growing chicks as recommended by Jukes ('39).

Data are presented in table 2 showing the pantothenic acid content of the whites and yolks of hens on the deficient diet and the values at various periods after the supplement was added.

Weight records of whites and yolks of all eggs were kept, and from these the pantothenic acid content per 100 gm. of egg (white plus yolk, neglecting the shell) was calculated. These values are given in column 5 of tables 1 and 2. The effect of the addendum is immediately reflected by an increase in the pantothenic acid content of both the whites and yolks. This

increase continued for about 18 days after supplementation was begun, then approached an approximately stationary level more than twice that in the eggs from hens on the stock diet. While the ratios of the pantothenic acid contents of the deficient diet to the stock diet to supplemented diet were 1:4.3:10.5, those of the eggs obtained from hens maintained on these diets were 1:4.8:9.8.

TABLE 2

Effect of supplementing deficient diet with pantothenic acid

NUMBER	DAYS ON PANTOTHENIC ACID	PANTOTHENIC ACID		WHOLE EGG (WITHOUT SHELL)
		White	Yolk	
		μg./gm.	*μg./gm.*	*μg./100 gm.*
64	Initial	0.34	7.3	254
	3	0.48	12.0	488
	8	1.90	34.0	1210
	18	2.20	100.0	3320
	24	3.20	92.0	3400
65	Initial	0.33	7.4	289
	4	0.52	11.0	417
	14	3.50	54.0	2070
	18	1.70	98.0	3470
	20	3.60	100.0	3870
66	Initial	0.60	12.5	467
	5	1.40	24.0	935
	6	2.00	27.0	1095
	14	1.90	63.0	3210

Within the limits of errors in sampling, assay, etc., these ratios are identical. It is thus evident that the pantothenic acid content of the egg is directly dependent upon the level at which pantothenic acid is fed.

SUMMARY AND CONCLUSIONS

Eggs from hens maintained since hatching on a diet low in pantothenic acid showed a markedly lowered pantothenic acid content. This increased rapidly when the diet was supplemented with synthetic pantothenic acid. From quantitative data secured, it is concluded that within the limits tested the

pantothenic acid content of the egg is directly proportional to that of the diet.

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BRIEF HISTORICAL NOTES ON MEAD'S CEREAL AND PABLUM

HAND in hand with pediatric progress, the introduction of Mead's Cereal in 1930 marked a new concept in the function of cereals in the child's dietary. For 150 years before that, since the days of "pap" and "panada," there had been no noteworthy improvement in the nutritive quality of cereals for infant feeding. Cereals were fed principally for their carbohydrate content.

The formula of Mead's Cereal was designed to supplement the baby's diet in minerals and vitamins, especially iron and B_1. How well it has succeeded in these functions may be seen from two examples:

(1) As little as one-sixth ounce of Mead's Cereal supplies over half of the iron and more than one-fifth of the vitamin B_1 minimum requirements of the 3-months-old bottle-fed baby. (2) One-half ounce of Mead's Cereal furnishes all of the iron and two-thirds of the vitamin B_1 minimum requirements of the 6-months-old breast-fed baby.

That the medical profession has recognized the importance of this contribution is indicated by the fact that cereal is now included in the baby's diet as early as the third or fourth month instead of at the sixth to twelfth month as was the custom only a decade or two ago.

In 1933 Mead Johnson & Company went a step further, improving the Mead's Cereal mixture by a special process of cooking, which rendered it easily tolerated by the infant and at the same time did away with the need for prolonged cereal cooking in the home. The result is Pablum, an original product which offers all of the nutritional qualities of Mead's Cereal, plus the convenience of thorough scientific cooking.

During the last ten years, these products have been used in a great deal of clinical investigation on various aspects of nutrition, which have been reported in the scientific literature.

Many physicians recognize the pioneer efforts on the part of Mead Johnson & Company by specifying Mead's Cereal and PABLUM.

Pablum is a palatable mixed cereal food, vitamin and mineral enriched, composed of wheatmeal (farina), oatmeal, cornmeal, wheat embryo, beef bone, brewers' yeast, alfalfa leaf, sodium chloride, and reduced iron.